The Physical and Chemical Characterisation of 3,4-Methylenedioxymethamphetamine Ecstasy Tablets
Implications for Users

Mifsud, Mario

Awarding institution:
King's College London

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Institute of Psychiatry

at The Maudsley

KING'S College LONDON

University of London

The Physical and Chemical Characterisation of 3,4-Methylenedioxymethamphetamine Ecstasy Tablets: Implications for Users

by

Mario Mifsud
B.Pharm (Hons.) (Melit.); MSc. (Lond.)

A thesis submitted to King's College London, Institute of Psychiatry, Department of Addiction, for the Degree of Doctor of Philosophy

Malta – 2013

I dedicate this work
to the loving memory of my late father Matthew and father-in-law Patrick,
may they rest in peace.

Acknowledgements

This thesis would not have been possible without the guidance and help of several individuals who in one way or another helped me achieve my dream.

I am heartily thankful to Dr. Kim Wolff, King's College London, Dr. Sue Jickells, University of East Anglia, Norwich and Dr. Janet Mifsud, University of Malta, my supervisors, for their advice, guidance, and support from the beginning of this research until the completion. .

Special thanks also go to Dr. Liberato Camilleri, University of Malta, who helped with my statistical problems, Dr. Andrew T. Kicman, King's College London, for the supply of the drugs samples and Professor Antonio Randazzo, University "Federico II" of Naples, for doing the ^{1}H NMR analyses.

I would also like to acknowledge those who were willing to share their bright thoughts with me, which were very fruitful for shaping my ideas for my research, who have been Dr. Les King, former head of the Drugs Intelligence Unit, Dr. Roumen Sedefov, European Monitoring for Drugs and Drug Addiction, Professor Reiner Schmid, New University Hospital of Vienna, Professor Alain Verstraete and Elke Devuyst, Ghent University Hospital, Dr. Kjell Andersson, Sweden Police Force, Dr. Istivan Ujvary, University of Budapest, and Mr. Bo Pallavicini, Europol.

Big thanks also go to my colleagues at the laboratory especially to the late Patrick Calleja, x-Chairman of the Malta National Laboratory, Godwin Sammut and Joseph Belli who helped during the SEM/EDX analyses. I also wish to express my gratitude to Neil Harrisson, Assistant Commissioner, and Norbert Ciappara, Superintendent, of the Malta Police for helping with my drug seizures and the Malta Party.

Last but not least my very dear wife Frances for her continuous encouragement, support and patience during these last six years. I also wish to thank all those who in any way had encouraged and helped me during my thesis.

CONTENTS

ABBREVIATIONS

^{1}H NMR	^{1}H nuclear magnetic resonance
3,4-MDP2P	3,4-(methlenedioxyphenyl)-2-propanone
5-HT	5-hydroxytryptamine (serotonin)
ANOVA	analysis of varianace
BZP	1-benzylpiperazine
(CIE) L*a*b*	Colour space specified by the International Commission on Illumination (CIELAB)
CHAMP	Collaborative Harmonisation Methods for Profiling of Amphetamine Type Stimulants
CMC	critical moisture content
CYP450	cytochrome P450
DA	dopamine
DCP	dibasic calcium phosphate
DMMDA	N,N-dimethyl-MDA
DPIA	di-(β-phenylisopropyl)amine
EDM	electronic dance music
EI	electron impact (ionisation)
EMC	equilibrium moisture content
EMCDDA	European Monitoring Centre for Drugs and Drug Addiction
EOCD	ecstasy and other club drugs
F254	fluorescent indicator at 254 nm UV
FTIR	Fourier transform IR
GBL	gamma-butyrolactone
GC	gas chromatography
HHA	4-dihydroxyamphetamine
HHMA	3,4-dihydroxymethamphetamine
HMA	4-hydroxy-3-methoxyamphetamine
HMMA	4-hydroxy-3-methoxymethamphetamine
HPLC	high performance liquid chromatography
ICH	International Conference on Harmonisation
IR	Infrared

KBr	potassium bromide
LSD	lysergic acid diethylamide
MA	methamphetamine
MBDB	N-methyl-1-(1,3-benzodioxal-5-yl)-2butanamine
mCPP	1-(3-Chlorophenyl)piperazine
MDA	3,4-methylenedioxyamphetamine
MDDMA	3,4-methylenedioxydimethylamphetamine
MDEA	3,4-methylenedioxyethamphetamine
MDMA	3,4-methylenedioxymethamphetamine
MeOD	deuterated methanol
MS	mass spectrometry
mt	metric ton
m/z	mass-to-charge ratio
NA	noradrenaline
RH	relative humidity
rpm	revolutions per minute
RQD	relative quartile deviation
RSD	relative standard deviation
Rf	retardation factor
Rt	retention time
SD	standard deviation
SEI	secondary electrons
SEM/EDX	scanning electron microscopy with energy dispersive X-ray analyser
SERT	serotonin transporter
SROs	safrole-rich oil
TB	tabletting
TFAA	trifluoroacetic anhydride
TIC	total ion chromatogram
TLC	thin layer chromatography
UNDCP	United Nations International Drug Control Programme
UV	ultraviolet
WHO	World Health Organisation

Abstract

The Physical and Chemical Characterisation of 3,4-Methylenedioxymethamphetamine / Ecstasy Tablets: Implications for Users

Aim: The physical and chemical characterisation of batches of 3,4–methylenedioxymethamphetamine (MDMA) / 'ecstasy' tablets seized in Malta were investigated to derive new information to be used for forensic intelligence purposes.

Methods: Thirty seizures containing 45 batches of 'ecstasy' tablets were investigated for their physical (logo, breakline, colour, shape, mass, diameter, thickness, hardness, friability and disintegration rate) and chemical characteristics over a 5 year period. Impurity profiling was carried out by GC-MS with additional chemical characterization by colour tests, TLC, FT-IR and SEM-EDX. MDMA enantiomer ratio was determined by TFAA derivatisation followed by GC-MS analysis on a chiral column. The effect of UV and fluorescent light, temperature (5, 15, 25, 35 and 40°C) and humidity (33 and 75% RH) on the physical features was assessed. 'Ecstasy' tablets from seizures (N = 172) at EDM parties by the police were analysed to determine the psychoactive substance content per tablet. Partygoers at an EDM party were interviewed to determine their drug-use, including 'ecstasy' tablets and party behaviour.

Results: Of the 45 batches examined 66.7% contained MDMA only as the main active ingredient, 6.7% contained mCPP, 4.4% BZP, 2.2% (1 batch) DPIA, 2.2% methanostenolone and 13% caffeine only. The majority of seized tablets were round in shape (91%) with logo (88.9%). The mean of means for mass of 237.2 mg (RSD 28.9%), diameter 8.09 mm (RSD 12.0%) and thickness 3.86 mm (RSD 19.1%). Of the 4 batches of tablets subjected to photostability testing (white, blue, green and orange colour), only the orange-coloured tablets showed a significant change in colour ($p < 0.05$, one-way ANOVA). The diameter of tablets stored at 15°C and 75% RH increased by 1.22 mm ($p < 0.05$, one-way ANOVA) and the thickness of tablets stored at 40 and 15°C respectively and 75% RH increased by 0.14 and 0.12 mm respectively ($p < 0.05$, one-way ANOVA). Chemical profiling determined that the Leuckart and reductive amination reactions were most probably used to synthesize the racemic MDMA and all tablets had a 50:50 ratio of R and S enantiomers. Lactose was the excipient mostly detected in tablets (40%). 'Ecstasy' tablets were the second most commonly confiscated drug at EDM parties (27.9%, n = 48 / 172) (cannabis being the most common), however our interviewed clubbers mostly (98.3%, n = 57) used alcohol.

Conclusion: Physical and chemical characterisation can help link or discriminate between batches of 'ecstasy' tablets. For intelligence purposes tablets should be stored away from sunlight (visible and UV light) and at low RH (≈ 25%) and temperature (≈ 25°C).

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Chapter 1

INTRODUCTION

The drug 3,4-methylenedioxymethamphetamine (MDMA), as described in this study, is used recreationally. Millions of people have taken MDMA tablets with the hope that they will feel love, empathy with others, and euphoria. Although some will take these tablets in small social gatherings [1], the majority of people try these tablets currently at Electronic Dance Music (EDM) events [2].

1.1. MDMA History and Recent Trends

1.1.1 MDMA: Production by Merck and subsequent research

MDMA was first synthesised in 1912 by a German chemist, Dr. Anton Köllisch [3]. Köllisch discovered the compound as an intermediate, during synthesis for haemostatics (compounds to stop the flow of blood) at the pharmaceutical company Merck [4, 5]. The compound MDMA, which was synthesised by the bromosafrole method, was patented in 1914 [3].

It was not until the late 1960s that MDMA was used recreationally when the free-thinking pop aficionados (New Age Seekers) started using this compound to achieve feeling of well-being and as an adjunct to spiritual pursuit [6]. MDMA appeared on the street in Chicago in the early 1970s where it was first detected in tablets [7].

By 1977 Shulgin introduced the drug to a retired psychologist Leo Zeff [5]. It was Zeff who in 1977 became the first psychotherapist to use MDMA as an adjunct to psychiatric treatment [8]. The apparent ability of MDMA to foster communication and increase empathy surprised Shulgin, Zeff, and other psychologists and psychiatrists [9]. By 1978

Shulgin together with Dr. Dave Nichols published the first human study on MDMA [4] concluding that the drug induced an easily controlled distorted state of awareness, with emotional and physical association [10]. During the 1978 and the mid 1980s, in the US, MDMA was introduced to psychotherapists to assist in psychotherapy, a process which was never sanctioned by the American Psychological Association or the Food and Drug Administration [11]. The media's reporting on the use of MDMA by psychotherapists as a psychocurative adjunct, a treatment to alter distress into eustress, increased the reputation of the drug [12].

At this time MDMA began to enjoy a slow expansion in recreational use [12]. In 1984 in California MDMA was given the street name "ecstasy" [3] and by 1986 underground laboratories were reported throughout the US [7]. An overview of the history of MDMA / ecstasy is given in Table 1.1 below.

1.1.2 Recognition of recreational use of MDMA

The recreational use of MDMA, which appears to have first occurred in the early 1970s [13], was moderate until the end of the decade [14]. By the early 1980s, the use of MDMA, being still legal, was promoted in some parts of the US as a "fun drug" and "good to dance to" because it was claimed that the drug could increase communication, emotional expression, empathy and prosocial feeling [8, 15]. When the drug reached recreational markets it was distributed and sold in US nightclubs [15]. During the mid-1980s the drug was exported, originally smuggled [15], from US discotheques to their counterparts in Europe [16, 17]. MDMA, soon gained popularity and started to be used at all-night electronic dance parties [15, 17] in clubs on the Spanish holiday island of Ibiza in the Mediterranean.

During the second half of the 1980s MDMA was fused with a distinct type of music and dancing called "acid house" on Ibiza [8]. This developed into the rave scene and other drugs such as lysergic acid diethylamide (LSD) and cannabis were popularised at all-

night dance parties [17]. Rave events and MDMA use also spread across the continent and tended to be secretive [18] all-night dance parties, that were held in underground

Table 1.1 History timeline of MDMA / ecstasy

Year	***Event***	***Literature***
1912	MDMA first synthesised and patent filed by Merch.	[3, 19]
1914	MDMA patented.	[3, 19]
1953/4	First animal study using MDMA (chemical warfare code EA-1475) and other seven other psychotropic drugs at the University of Michigan under classified contract with US Army. Research was published in 1973.	[8, 13, 19, 20]
1959	Merck Chemist, Dr Wolfgang Fruhstorfer, who was interested in the production of new stimulants resynthesised and worked with MDMA	[3, 5]
1960	Biniecki and Krajewski, published a paper describing the synthesis of MDMA by the conversion of safrole to the beta-bromopropane with HBr, and its subsequent conversion with alcoholic methylamine to MDMA	[21]
1965	Theodore Shulgin synthesised MDMA from MDA via N-formyl-MDA (Leuckart reaction) and from 3,4-MDP2P using aluminium amalgam	[3, 22]
1970	First detection of MDMA in tablets in Chicago	[3]
1970s - 1980s	Mid 1970s and 1980s legal use of MDMA in psychotherapy.	[20]
1980s	Popular use of MDMA starts at the early part of the decade in the US	[20]
1978	First MDMA studies in humans by Shulgin and coworkers	[3, 8]
1984	MDMA street name ‘ecstasy’ was coined in California	[3]
1984	First reference to MDMA in an article published in “The Face” in the UK	[23]
1984-85	Start of MDMA tablets in US	24
1986	Underground laboratories were reported all through the US	[7]
late 1980s	‘Ecstasy’ drugs first appeared in the UK as capsules	[25]
1988	Britain’s “Summer of Love” – increased MDMA consumption	[20]
1987-91	Rave scene started together with use of ‘ecstasy’ in the UK	[23, 26]

locations or in clubs with a number of attendees taking MDMA. All this created a sub-culture and an explosion in the recreational use of MDMA [27].

In the UK in the early 1980s MDMA was a relatively new recreational drug [28]. By 1987 rave parties in London had acquired such popularity among youths and adolescents that they had outgrown most dance clubs [18]. Many thousands started to attend rave festivals [4]. By 1990s in the UK MDMA started to feature prominently in surveys as a recreational drug [28]. However, the recreational use of 'ecstasy' is not well documented in the UK [28]. Although non-human research was conducted on the effects of MDMA this provided very little information on the drug consumption by users at dance parties in the UK [28]. By the late 1980s and early 1990s the phenomenon of rave parties with the use of MDMA was exported back to the US and spread globally [26]. The increase in the use of MDMA was so dramatic that by the end of the 1990s global seizures of MDMA were still increasing (global seizures 1999 about 22 from 5.6 million in 1998) [15, 29].

1.1.3 MDMA / ecstasy tablets production in Europe

In the Netherlands during the end of the 1980s most MDMA tablets and powder were imported from Spain and the US [15, 30]. Spain, which had rudimentary laboratories, also supplied Ibiza. The MDMA powder that was imported to the Netherlands from the US was locally tabletted [15, 30]. When MDMA arrived in the Netherlands, at the end of the 1980s, users were frequently "home users" but by the end of the decade the "mainstream" use of MDMA was at parties and raves [31].

In the UK in the late 1980s the use of MDMA was minimal because the process for producing the drug was relatively expensive [32]. However, by the early 1990s the 'ecstasy' laboratories in the UK were highly commercial, thus the price of MDMA tablets fell sharply from £ 15-20 in 1987 to £ 7 in 1996 [32].

The initial small 'ecstasy' laboratories in the Netherlands were set up by people taking the drug at parties and raves, while the 'ecstasy' business was started from the long-established Dutch leading role in manufacture and trafficking of amphetamines [31]. However, by the spring of 1990 the first professionally manufactured ecstasy tablets appeared on the Amsterdam market [30].

Most of the MDMA production in Europe during the 1990s occurred in the Benelux countries of Belgium, the Netherlands, and Luxembourg [33]. This was mainly due to the well established precursor chemical routes and to the ease by which these chemicals were accessed. The transportation of MDMA to consumer countries was not a problem, owing to the ease of access to international air and rail transportation routes [12, 33]. By the mid 1990s ecstasy (MDMA) tablets that were supplied to rave parties all over Europe came from the Netherlands [30]. This made Europe a major source of global ecstasy (MDMA) supply [15] when the use of ecstasy was rising globally [33]. Moreover, by the late 1990s some ecstasy tablets supposed to contain MDMA were found to contain other psychoactive phenethylamines, such as 4-bromo-2,5-dimethoxyphenethylamine [34]. However, while most of the 'ecstasy' tablets still continued to contain MDMA, manufacture of these tablets remained primarily in Europe [15].

During the period 1999 - 2001, 75% of all seized clandestine laboratories producing 'ecstasy' tablets were from the Netherlands and 14% from Belgium and more than half of all 'ecstasy' end-products and almost 90% of all 'ecstasy' precursors were seized in Europe [35]. The Netherlands was considered by most law enforcement and international drug control agencies as the world's major production and trafficking centre for synthetic drugs [31]. For the 'ecstasy' and amphetamine market, the Netherlands was similar to Colombia as the foremost cocaine manufacture [31].

1.1.4 Lower MDMA content in tablets and the emergence of designer drugs and 'legal highs'

Change in the contents of 'ecstasy' tablets sold on the European market had occurred during the last decade [15]. In 2005 most (70% or more) of the 'ecstasy' tablets contained MDMA or its analogues, 3,4-methylenedioxyethylamphetamine (MDEA) and MDA, as the only active substance. However, during the same year other psychoactive substances, not under international control, such as benzylpiperazine (BZP) and 1-(3-chlorophenyl)piperazine (mCPP) [36] were also detected in 'ecstasy' tablets [15]. Although by 2007 most of the European countries reported that their seized 'ecstasy' was sourced from within Europe [36], the 'ecstasy' market in Europe had already started to

undergo a significant transformation in the contents of 'ecstasy' tablets. This was mainly caused by reduced availability in the primary precursor for MDMA production, 3,4-(methylenedioxyphenyl)-2-propanone (3,4-MDP2P), because of control measures in production, trade and export [37].

In the UK similar developments were happening with regard to 'ecstasy' tablet scenario in Europe. Going back to 2002 till 2005 the number of 'ecstasy' seizures in the UK had stabilised before increasing again in 2006/07 (Table 1.2) [38]. Between 2002 and 2006/07 an average of about 5.1 million 'ecstasy' tablets were seized annually [38]. By 2008 in the UK only 51% of 'ecstasy' tablets were found to contain MDMA, a fall from 73% in 2007. The mean MDMA content in the tablets was almost half (33.1%) of what was detected in 2003 (64.5%) [39]. From 2008 until 2012 in the UK both the number of

Table 1.2 The UK number of seizures of 'ecstasy' tablets and number of tablets seized from 2002 till 2011 [38].

Year	2002	2003	2004	2005	2006/07	2007/08	2008/09	2009/10	2010/11	2011/12
Ecstasy Seizures	6,960	6,401	6,190	6,634	8,141	7,148	5,197	3,712	2,524	3,168
Tablets (000s)	4,132	6,899	4,740	3,019	6,685	965	547	171	371	656

'ecstasy' seizures and tablets had drastically decreased, with the year 2009/10 registering the lowest-ever level for both ecstasy seizures and tablets (Table 1.2) [38]. The drastic decrease in number of 'ecstasy' tablets seized in the UK during 2008 - 2011 (average 440 thousand annually, Table 1.2 [38]) seemed to have been counterbalanced by an increasing trend towards new substances termed 'legal highs' (unregulated new psychoactive substances [40]) as an alternative to 'ecstasy' [39].

The same trend happening in the UK with regards to the decrease in 'ecstasy' tablets and the increase in legal highs was happening in other European counties [15]. As this was happening, the availability of' 'ecstasy' tablets containing MDMA continued to decrease [15]. The 'ecstasy' tablets were substituted by synthetic cannabinoids, different amphetamines (e.g. fluoroamphetamine), cathinone derivatives (e.g. mephedrone,

methylone), and others, in tablets and powder forms [37]. A large number of ecstasy production sites were found to be tabletting mephedrone [41]. However, by the end of the last decade there seemed to be resurgence in 'ecstasy' market (Table 1.2). This partial resurgence may have been caused by a new pre-precursor chemical 3-[3′4′(methylenedioxy)phenyl]-2-methyl glycidate to the precursor 3,4-MDP2P, which was found in underground laboratories at a production site in the Netherlands to produce MDMA [15, 42].

1.2 The Malta Drug Situation

The Maltese archipelago (Figure 1.1) is located in the Mediterranean Sea and lies just south of Sicily and the northern most point of Africa. Malta consists of three main, inhabited islands: Malta, Gozo and Comino. The three islands cover together an area of 316 km^2 [45], have territorial waters that extend to 9.65 km from the coast, comprising an area of about 3000 km^2 [46]. Malta is considered to be one of the world's smallest and

Figure 1.1 The Maltese archipelago [43, 44].

most densely populated countries, having a population of about 414,000 [45]. The largest island of the group, Malta, is made up of small towns and villages, with the capital, Valletta, on the north east part of the island. The Island has two official languages, Maltese and English, with Maltese being the national language [45]. Malta became a member of the European Union (EU) in 2004, and a full member of the Schengen area in 2008. It has numerous connections to Europe and Africa due to daily flights and ships which call at its large commercial port. Flights from the Netherlands and Spain are considered high risk flights for the importation of 'ecstasy' tablets (N. Harrison, 2012, personal communication, Assistant Commissioner, Malta Police, interview, 12 June).

1.2.1 Importation of illegal drugs

The illicit drug problem in Malta, which was estimated to be the eighth-highest for problem drug use in the EU according to the 2010 World Drug Report [36], is normally limited to the sale and use of imported drugs. No evidence has ever been found to indicate that the production or processing of MDMA has taken place on the Island. Although there are a number of generic pharmaceutical firms operating on the Island there is no evidence that precursors or chemicals used in these firms have been diverted for illegal production of drugs. All illegal drugs, with the exception of few hundred cannabis plants, are imported to the Island from Europe and North Africa for local consumption (N. Harrison, 2012, personal communication, Assistant Commissioner, Malta Police, interview, 12 June). Cannabis was, and still remains, the most popular drug of abuse in Malta [47] and, while some cannabis plants are grown on the Island due to the good climatic conditions, cannabis resin (hashish) and herbal cannabis (also referred to as marijuana, grass or weed) is mainly imported from Morocco (N. Harrison, 2012, personal communication, Assistant Commissioner, Malta Police, interview, 12 June). Heroin, cocaine and 'ecstasy' are also commonly imported to Malta.

The 'ecstasy' tablets that were seized in Malta from 1995 till 1999 were small in number, less than 500, but from 2000 onward the number of 'ecstasy' tablets seized sharply increased reaching the highest in 2006 when 67,182 tablets were seized. The

number of ‘ecstasy’ tablets seizures was high until 2011 when it drastically declined (Table 1.3).

Table 1.3 The number of ‘ecstasy’ tablets seized in Malta between 1995-2012 (N. Harrison, 2013, personal communication, Assistant Commissioner, Malta Police, email, 23 January).

Year	1995	1996	1997	1998	1999	2000	2001	2002	2003
Tablets	513	687	247	153	459	5,192	2,458	11,012	8,695
Year	2004	2005	2006	2007	2008	2009	2010	2011	2012
Tablets	6,071	17,273	67,182	30,259	13,677	24,832	36,187	2,143	1,080

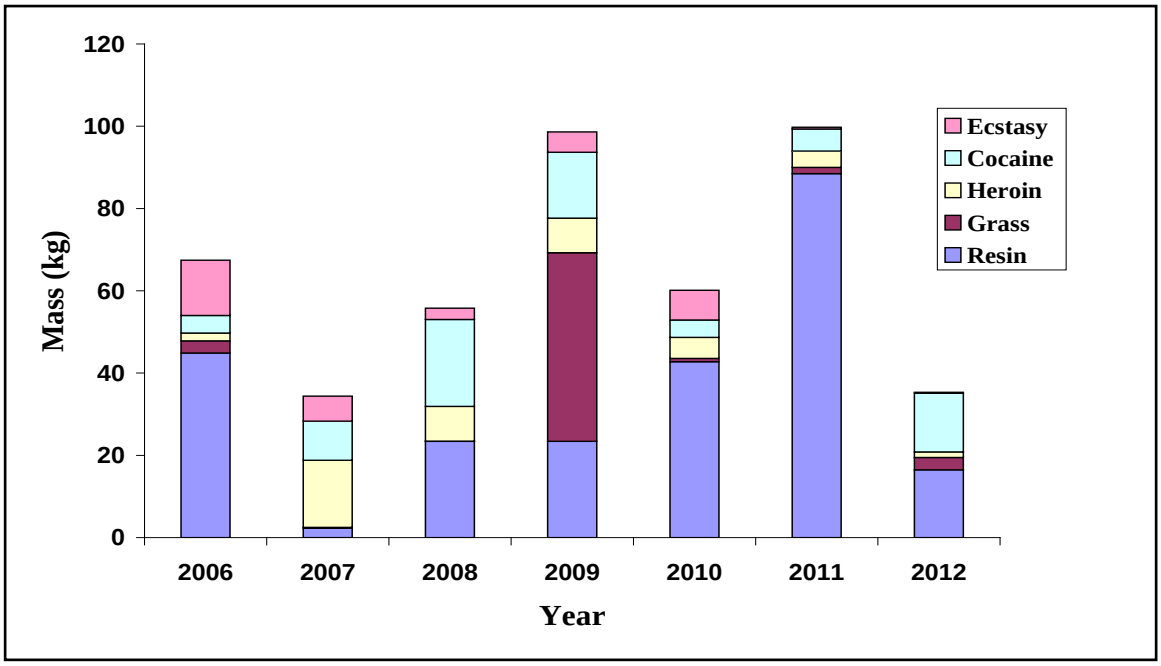


Figure 1.2 Illegal drug seizures of cannabis (resin and grass); cocaine, ‘ecstasy’ and heroin in Malta between 2006 – 2012 (N. Harrison, 2013, personal communication, Assistant Commissioner, Malta Police, email, 23 January), (*Note: cannabis grass weight for 2009 and cocaine weight for 2012 were divided by 10).*

Between 2007 - 2009 and 2011, when ‘ecstasy’ tablets seizures declined heroin and cocaine were the second most commonly seized drugs (after cannabis) on the Island. In 2012 in Malta when ‘ecstasy’ tablets seizures were very low, cocaine was the most seized drug (142.9 Kg) on the Island (Figure 1.2) (N. Harrison, 2013, personal communication, Assistant Commissioner, Malta Police, email, 23 January). Heroin is mainly imported from Eastern Mediterranean Countries and North Africa. However, during the last five

years there were cases where cocaine and heroin were imported from the African Western Hemisphere, Eastern European countries and South America. Ecstasy tablets and small amounts of amphetamine were, and still are, mainly imported from Europe, mainly from the Netherlands [47]. Most recently from 2009 - 2012 new legal highs (e.g. mephedrone) and herbal highs (synthetic cannabinoids) have also been identified in Malta (N. Harrison, 2013, personal communication, Assistant Commissioner, Malta Police, interview, 8 February).

1.2.2 MDMA / ecstasy use within party settings in Malta

The rave / dance party culture began in Malta in the early 1990's when a small group of people, who had close ties with the London rave scene, started organizing rave parties on the Island [48]. By 1992 rave parties in Malta were opened to the public. These parties were called S.I.N., Sex Is Natural, parties by the promoters of these events but stopped due competition within a year. It is claimed that by 1994 the popularity of rave parties in Malta was very high [49]. These were the authentic rave parties which were normally held outdoors in illegal locations, outside the legitimate entertainment establishment [52]. There is no published data about the number of rave parties and attendees that attended these events during those years in Malta. When authentic raves declined these parties moved to legally operated venues. With the decline in raves parties, which was mainly caused by law enforcement, commercial forces and generational schism, where aging ravers were not replaced by younger generation [50], the commercial EDM events took over.

Today large parties are organized in summer; the peak holiday season and disc jockeys (DJ's) such as David Guetta, Paul Oakenfold, Carl Cox, and Tiësto, are invited to play. However, there is little data of the number and nationality of attendees and the type of illicit drugs, if any, that are consumed at these venues.

A study which was conducted in Malta in 2002 showed party goers who used 'ecstasy' took the drug between the ages 16-19 years [48]. The European School Survey Project on

Alcohol and other Drugs studies conducted in 1999 and 2003 respectively in Malta on about 5,000 students aged between 15-16 years, showed that in 1999 and 2003, 2% and 1.2% respectively of those surveyed have taken 'ecstasy' [51, 52]. By 2007 the annual prevalence of 'ecstasy' use in Malta for students in the 15-16 age groups had risen to 4% [53].

According to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA, 2010) the prevalence of 'ecstasy use' in the Maltese general population between 2004 and 2007 for those aged 15-64 years was 0.7% (EU range 0.3-8.3%) for life time use and 0.2% (EU range 0.1-1.6%) for last year use [54]. This was considered low by EMCDDA. Furthermore, the prevalence for lifetime 'ecstasy' use for Maltese aged 15-34 years between 2004 and 2007 was 1.4% (EU range 0.6-12.7%) [54]. Although, the 2007 'ecstasy' use for the Maltese population (15-64 years) was well towards the bottom of the EU range that for students in the 15-16 age group was relatively high (4.0%) when compared to 0.2% for the general population. In 2009, a study was conducted at the University of Malta on 494 undergraduate students (mean age 17.2 years, range about 16-27 years) revealed that 6% of the students had used ecstasy [47]. In 2009 the Maltese Parliamentary Social Affairs Committee issued a statement showing its concern about the use of illicit drugs at parties and that 'ecstasy' users were not seeking treatment for 'ecstasy' related induced psychiatric syndromes [55].

There is a lack of research on drug use at EDM events that are held in Malta [48]. Research by others in Europe and the US has shown widespread drug use by attendees at dance events [19, 56, 57]. This information is not currently available for the country of Malta. To this end two studies were taken up to try and investigated the licit and illicit substances use and the behaviour of attendees at EDM parties in Malta. Previous studies focused on the general population, secondary school and university students to determine the prevalence both licit and illicit substance use. The studies conducted in this research were different from what had gone before because these concentrated on partygoers at EDM parties where illicit substance use is largely hidden.

1.3 MDMA: Chemistry and Structure-Activity Relationship

Illicit tablets containing MDMA hydrochloride are usually taken orally in a tablet form having a characteristic logo, and less commonly as white powder or capsules [58]. This substance belongs to a family of drugs that has the basic phenethylamine structure which is found in other sympathomimetic agents, such as amphetamine, methamphetamine (MA) and catecholamines. The phenethylamines, which possesses a broad range of central nervous system actions, unlike other class of drugs can produce a wide spectrum of central nervous system effects by a small change in their structure [59]. MDMA, being a ring substituted derivative of phenethylamine, is a psychoactive drug which is a completely synthetic substance that does not exist in nature. It has been classified in the therapeutic class of compounds, the entactogens, because of its pharmacological properties and mechanism of action. MDMA is structurally related to the hallucinogenic MDA, being its N-methyl analog, and to the stimulant MA, being its 3,4-methylenedioxy-analog [60]. Several structure-activity relationships clearly differentiate MDMA from other hallucinogenic amphetamines.

1.3.1. Chemistry

The International Union of Pure and Applied Chemistry (IUPAC) systemic name for MDMA is 1-(1,3-benzodioxl-5yl)-N-methylpropan-2-amine [61] but in the literature it is more generally referred to by the older chemical name 3,4-**M**ethylene**D**ioxy**M**eth**A**mphetamine, from which the acronym MDMA ($C_{11}H_{15}NO_2$ – CAS – 42542-10.09) is derived. Other chemical names include methylenedioxy-methylamphetamine and N, α-dimethyl-3,4-methylenedioxy-phenylamine. Since MDMA is structurally related to MA, it is also known by names related to MA, such as N-methyl-3,4-methylenedioxyphenylisopropylamine and N-methyl-3,4-methylenedioxyamphetamine [62]. MDMA, a secondary amine compound having a molecular weight of 193.25 a.m.u., has a chiral centre giving rise to two enantiomers, each having different degrees of central nervous system activity and also different affinities to the enzyme responsible for its breakdown [62]. The base compound, which

at room temperature is colourless oil with a boiling point of 100 - 110^{o}C, is insoluble in water [62, 58]. Similarity exists between the aromatic methylenedioxy substituent of MDMA and the substance found in the oils of the natural products safrole and myristicin [63]. The most common salt of MDMA is the hydrochloride ($C_{11}H_{16}NClO_2$ – CAS-64057-70-1), which is a white or off-white powder or crystals. The salt, which is soluble in water, has a melting point of 148 – 149^{o}C [58, 62].

1.3.2 Structure-activity relationship (SAR)

The SAR of MDMA, which is the relationship between the chemical and its biological activity, is dependent on its two optical isomers, which belong to a family of drugs that has the basic phenethylamine structure. MDMA as a biologically active phenethylamine compound is typical and has one isomer more active than the other [64]. The phenethylamines (Figure 1.3) consist of a group of compounds with a stimulant effect (e.g. amphetamine) whereas the S-(+)- enantiomer is more active, a group of compounds with action similar to hallucinogens (e.g. 2,5-dimethoxy-4-methyl-amphetamine) having a predominant R-(-)- enantiomer [65], and in between a whole spectrum of biologically active compounds [65]. Within all this MDA stands alone because its two biologically active optical isomers have different effects at nearly the same dose. The R-(-)-MDA has a hallucinogenic effect, while the S-(+)-MDA has more like an amphetamine effect [64]. The addition of a methyl group to the nitrogen atom (N-methylation) of MDA, producing MDMA, makes the molecule too large to fit the serotonin 5HT$_2$A receptor of the brain [4], thus reducing or eliminating the hallucinogenic activity of MDA [64, 65]. It appears that these effects made MDMA more desirable than MDA [66]. Furthermore, if the α-methyl of a hallucinogenic compound is extended to an α-ethyl all hallucinogenic activity is abolished. Hence the ethyl homologue of MDMA, N-methyl-1-(1,3-benzodioxol-5-yl)-2-butanamine (MBDB), which combines the N-methylation and α-ethylation show no hallucinogenic effects [64]. In terms of the biological effects of MDMA the N-methylation of MDA enhances the lipohilicity of the new molecule MDMA thus facilitating the rapid entry into the brain and producing a faster in onset of effects but with shorter lasting than MDA [4]. The N-methyl group in the MDMA molecule has no

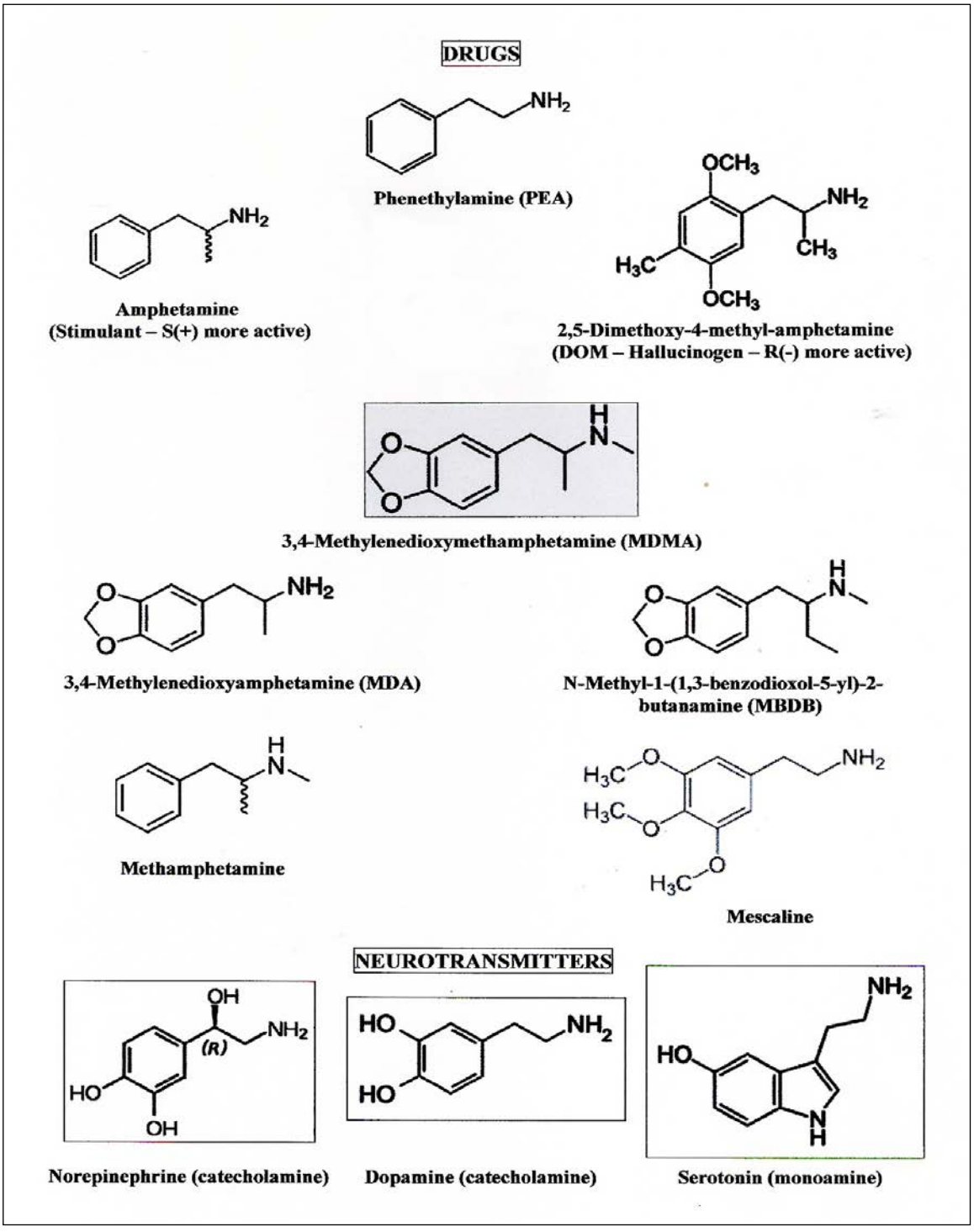


Figure 1.3 Chemical structures of drugs having the basic phenethylamine structure and neurotransmitters.

effect on the reuptake pump that transports MDMA into neurons that make use of serotonin and dopamine (DA).

In the literature it is sometimes claimed that MDMA is a sort of a mix between amphetamine and mescaline [66, 67], a mixture of central stimulant and psychedelic [57, 59, 68-70] and that the compound is related to hallucinogen S- type substances [27, 71]. While MDMA and MA have a similar backbone, the S-(+)- MA enantiomer is more potent than S-(+)-MDMA enantiomer and able to release the stored neurotransmitter DA from DA-containing neurons. The feeling of euphoria and wellbeing produced by MDMA could also be related to its actions on DA systems which in such a case acts similar to MA [4]. Both compounds can bring about the release of other neurotransmitters such as noradrenaline and serotonin but the release of serotonin by MDMA is much more pronounced than that of MA. Whereas the only relationship between MDMA and mescaline is that they are both phenethylamines. The oxygen atoms on the mescaline ring do not have the same biological effect as the methylenedioxy (O-CH2-O-) group on the phenyl ring of MDMA, which is largely responsible for the release of serotonin [6]. Moreover, the lack of hallucinogenic activity of MDMA, except at very high doses [12], continues to make these compounds pharmacologically different.

The stimulant effect of both MDA and MDMA, although MDMA is less potent than MDA, is achieved by the ability of their S-(+)- isomers to block the reuptake of DA [64, 72]. Both the hallucinogen 2,5-Dimethoxy-4-bromoamphetamine and the entactogen MBDB have no such ability, thus indicating that the entactogenic effect is not mediated through the dopaminergic pathway [64]. However, the compounds MDA, MDMA and MBDB which are all able to release serotonin (unlike the hallucinogen 2,5-dimethoxy-4-methylamphetamine), are also potent serotonin reuptake inhibitors. This indicates that the primary mode of action of entactogenic compounds is the ability of their S-(+)-isomers, which is the most active, to release serotonin and block its reuptake into the nerve terminals [64].

1.4 MDMA: Pharmacology and Toxicology

To better understand the desired and undesired effects of MDMA on the user a brief review of the pharmacology of the drug is given. The effects of MDMA are dependent on the dose, the frequency and duration of use. Serious or fatal toxicity that might occur if the drug is used at higher doses by sensitive individuals that could suffer from adverse effects such as psychoactive, cardiovascular, and hepatic among others, or if used with other illicit drugs, is described separately [73-75].

1.4.1 Pharmacology of MDMA

MDMA is metabolized by two main pathways [76]. The first pathway involves N-dealkylation, and O-demethylenation, while the second pathway involves O-methylation. MDMA also causes the release of serotonin (5-HT), DA and noradrenaline (NA) in the central nervous system [73].

1.4.1.1 Pharmacokinetics

MDMA is usually taken by ingestion and is absorbed from the small intestinal tract rather than the stomach. It reaches its peak concentration in plasma about 2 hours after oral administration [73, 77]. The metabolism of MDMA involves the N-demethylation by CYP3A4 to its main active metabolite MDA (Figure 1.4) [76, 78, 79]. Since MDA is pharmacologically active the duration of action of MDMA may be somewhat longer [73]. Both MDMA and MDA are O-demethylenated to 4-hydroxy-3-methoxymethamphetamine (HHMA) and, 4-dihydroxyamphetamine (HHA) respectively, which are regulated by the enzymes in the liver (CYP2D6) [76, 78, 79]. Individuals with genetic variations of the CYP2D6 enzyme (slow or rapid metabolisers) may have variable concentrations of the parent compound MDMA and its metabolites [74]. Furthermore, HHMA and HHA are both O-methylated by catechol-O-methyltransfarase mainly to 4-hydroxy-3-methoxymethamphetamine (HMMA) and 4-hydroxy-3- methoxyamphetamine

(HMA) respectively [76, 78, 79]. The conjugated glucuronide and sulphate of the four metabolites, particularly HMMA and HMA, are excreted in the urine [76].

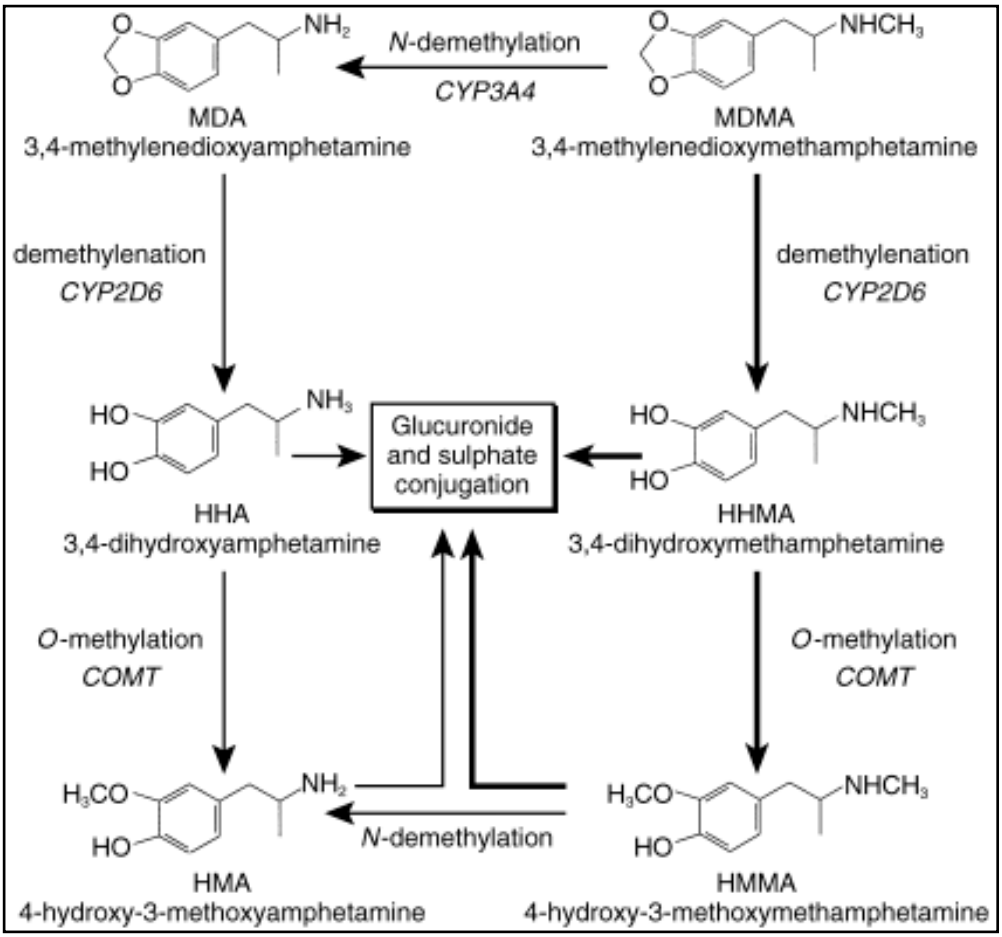


Figure 1.4 Metabolism of MDMA [76].

Potent 2D6 inhibitors, such as fluoxetine, paroxetine (both antidepressants of the selective 5-HT reuptake inhibitor class), and cocaine, may block the main metabolic pathway and therefore increase the concentration of MDMA [64]. MDMA has non-linear pharmacokinetics, when regular users take several doses at one time large increases in blood and brain concentration of the drug occur [73, 76], thus small increases in dosage may increase the risk of toxicity [73]. Since the half-life of MDMA is in the order of 8 hours and it takes about 5 half-lives to remove the drug from the body it is estimated that it may take about 40 hours for over 95% of the drug to be cleared from the

body [73]. This could be the reason for the after-effects of the drug, after one to two days of its use, because some of its metabolites, such as MDA could still be pharmacologically active [73].

It is reported that the clandestine synthetic procedures usually employed in the preparation of MDMA are not stereoselective and the hydrochloride salt, employed in the clandestine manufacture of tablets, is a racemic mixture, of the S-(+)- and R-(-)-enantiomers [80]. However, in this study MDMA from seized ecstasy tablets were investigated to determine if illegal chemists were producing a racemic or chiral specific MDMA tablets.

The activities of the different MDMA stereoisomers were reported by Anderson et al. [81] and confirmed by different studies later on [82–85]. All studies confirmed the higher amphetamine-like activity (toxicity – mydriasis and jaw clenching) of the S-(+)-enantiomer [82–86] compared to the R-(-)- enantiomer where some studies suggest to have the mescaline or LSD-like hallucinogenic properties [73]. Differences in the pharmacokinetics of the stereoisomers of MDMA were demonstrated after rats were dosed with racemic MDMA given intravenously or subcutaneously [87]. All dose groups showed a greater area under the blood curve for R-(-)-MDMA than that of the S-(+)-MDMA, an enantioselective metabolism, where the S-(+)- enantiomer is metabolized faster than the R-(-)- enantiomer [62, 88]. Studies have also been done in humans and have demonstrated similar stereospecific differences in the biotransformation of MDMA, even though studies were limited to a few individuals [89–92].

1.4.1.2 Pharmacodynamics

The action of MDMA in the central nervous system is complex affecting several molecular sites. The major effects are on the 5-HT pathways but it also affects two other major transmitter systems in the brain, which are the DA and NA. MDMA increases the release of the monoamine neurotransmitters 5-HT, noradrenalin and to a lesser extent DA from the respective axon terminals [73]. The 5-HT receptors mediate the effects of

serotonin, as an endogenous ligand, and also of a number of pharmaceutical and psychoactive drugs, such as MDMA.

MDMA, does not directly release 5-HT but it enters the serotonergic neurones via the serotonin transporter (SERT) and reverses the normal direction of monamine travel thus inducing the release of 5-HT (Figure 1.5) by binding and blocking the reuptake of 5-HT [93**,** 94]. Inhibition by MDMA contributes to greater increases in 5-HT than direct

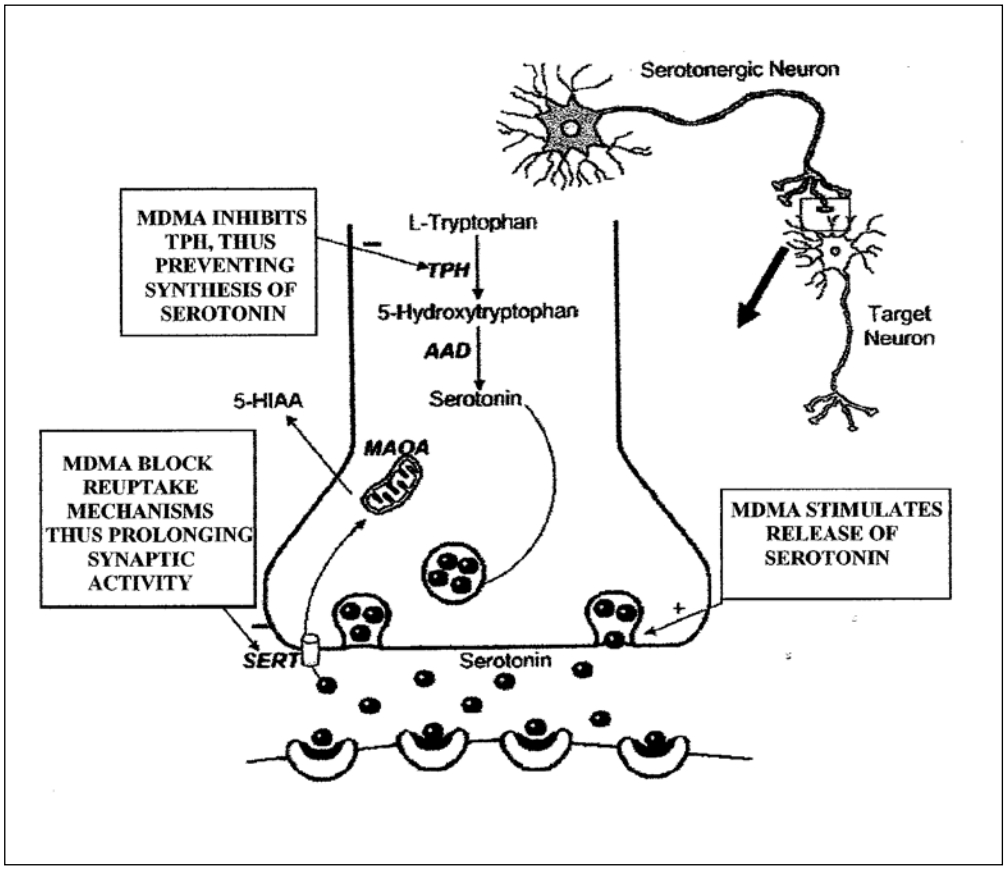


Figure 1.5 Schematic diagram showing the serotonergic synapse and the target for MDMA. Symbol: stimulatory effect (+); inhibition or blockade (-) (adopted from Ayala, 2009 [101].

release and the stimulant effects produced by MDMA are the indirect stimulation of 5-HT_{1B} (it induces presynaptic inhibition and behavioural effects) and 5-HT_{2A} (the main excitatory receptor subtype among the G protein-coupled receptor 5-HT) receptors [94, 95]**.** The emotional closeness, euphoria and sensory delight attributed to the use of

MDMA are caused by this 5-HT boost [74]. Some acute adverse effects caused by MDMA by decreasing the 5-HT reuptake (SERT) binding include increased heart rate and blood pressure, tremor, sweating and hyperthermia [74].

A similar, but weaker, action is also exerted on the reuptake of DA but the concentrations of MDMA required to induce DA release is 10 times higher than that needed to induce 5-HT release [96]. MDMA produces DA release and uptake inhibition by the DA transporter, and by noradrenergic neurons via the NA uptake pump [97–100]. MDMA inhibition of monoamine oxidase B prevents metabolism of DA [102]. It is also reported that MDMA displaces NA from adrenergic nerve terminal endings and may also be involved in the competitive blockade of NA transporter [103]. The characteristic MDMA effects, such as euphoria, altered perception, relaxation, and decrease in negative affect and defensiveness [104], of MDMA are attributed to the net release of 5-HT and possibly DA, while the physical effects (such as increase in wakefulness, sense of energy, decrease in fatigue and sleepiness [73]) are credited to the increased release of NA [73]. The S-(+)- enantiomer of MDMA is more potent than R-(-)- enantiomer in producing the prosocial characteristic effects that are credited to the drug [73].

1.4.2 Toxicity of MDMA

Although rare, MDMA use has been linked to serious toxicities and death [105]. The most profound adverse reaction to MDMA is hyperthermia and hyperpyrexia, with body temperature reaching as high as 44^{o}C usually followed by multiple organ failure. The majority of these adverse reactions occur at raves or nightclubs [106]. Other adverse reactions, which are caused by MDMA induced excess intrasynaptic 5-HT, can be grouped together under the heading of ‘serotonin syndrome’ which include behavioural hyperactivity, mental confusion, agitation, hyperreflexia, hyperpyrexia, tachycardia, shivering, clonus, myoclonus, ocular oscillation and tremor [107] and syndrome of inappropriate antidiuretic hormone secretion (SIADH) [108].

The severity of symptoms caused by the ingestion of ‘ecstasy’ tablets shows marked

variability in response suggesting individual pattern of metabolism, possibly genetically determined due to the highly polymorphic CYP2D6 gene [75], where 'slow metabolisers' are genetically predisposed to more severe MDMA toxicity [109]. In cases of death, involving MDMA, it was typically found that the deceased took several different drugs (prescribed and non-prescribed) with ecstasy, however death also occurred when ecstasy tablets containing MDMA were taken [110]. Severe Depression that could cause suicide could also be induced by MDMA [73]. Moreover, motor vehicle accidents were reported where drivers and pedestrians were affected by MDMA [73].

The toxicity could be further exacerbated with the use of other legal and / or illegal drugs that may interact with this substance. Some ecstasy users may concurrently take drugs in order to enhance and prolong the pharmacological effects (e.g. cocaine, known as 'bumping up', other amphetamine derivatives, caffeine) or to counteract undesired side effects (e.g. moclobemide - a reversible monoamine oxidase inhibitor drug, cannabis), such as insomnia, depression and anxiety [111, 112]. If MDMA is taken with cocaine and / or amphetamine it may increase the likelihood and severity of the serotonin effect [74], while with caffeine it promotes hyperthermia and serotonergic loss [113], and with moclobemide fatal serotonin toxicity could be caused [111]. Furthermore, if MDMA is taken with cannabis various psychological problems, such as impulsiveness, anxiety, somatic complaints, obsessive compulsive patterns, and psychotic behaviour, together with interpersonal sensitivity, depression, and paranoid ideation, could be caused [112]. What is clear is that the recreational use of 'ecstasy' is not always safe especially when taken with other drugs.

1.5 Synthesis of MDMA

The clandestine manufacture of MDMA requires chemical synthesis. Laboratories where the drug is synthesised range from improvised operations, where simple processes and makeshift apparatus are utilised, to sophisticated operations where advanced equipment and facilities are used [114].

1.5.1 Precursor chemicals used in MDMA synthesis

Precursor chemicals that are used for MDMA synthesis include safrole (3,4-methylenedioxyallybenzene), (some times in the form of safrole-rich oils – (SROs)), isosafrole (3,4-methylenedioxypropenylbenzene), piperonal (3,4-methylenedioxybenzaldehyde), and 3,4-MDP2P, which is the key precursor for MDMA synthesis. These compounds, which are all under international control, have also legitimate uses e.g. in perfumery and the food industry.

The SROs are the main raw materials for the manufacture of safrole for commercial purposes. Safrole-rich oil tree species that are found in North and South America and in East and South-East Asia grow naturally and / or are cultivated for commercial purposes [115]. SROs are distilled from the timber, root and stump and yields range between 1% and 3.5% [115].

The most common synthetic route for the production of MDMA is from the precursor 3,4-MDP2P. One litre of 3,4-MDP2P can yield more than 10,000 ecstasy tablets [116].

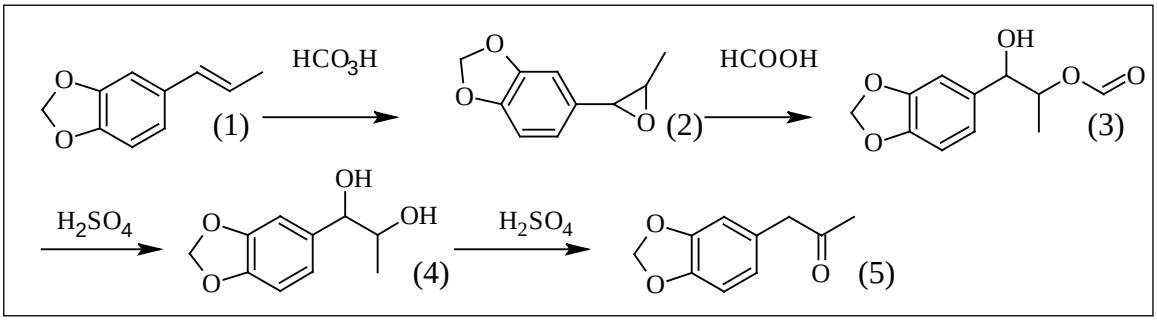


Figure 1.6 3,4-MDP2P synthesis by isosafrole oxidation (Note: (1) isosafrole, (2) isosafrole epoxide, (3) isosafrole monoformyl glycol, (4) isosafrole glycol and (5) 3,4-MDP2P) [118].

To get 3,4-MDP2P a natural source of safrole is required, which needs to be isomerised to isosafrole by heating with sodium hydroxide (NaOH) or potassium hydroxide (KOH) [117].

The two most probable routes of synthesis of 3,4-MDP2P in clandestine production are by oxidizing isosafrole in an acid medium (Figure 1.6) or by reduction of 1-(3,4-methylenedioxyphenyl)-2-nitropropene, which is prepared by condensation of piperonal. The common citation of the synthesis of piperonal is by oxidation from isosafrole with ozone (O_3) [119] and nitroethane (Figure 1.7) [119].

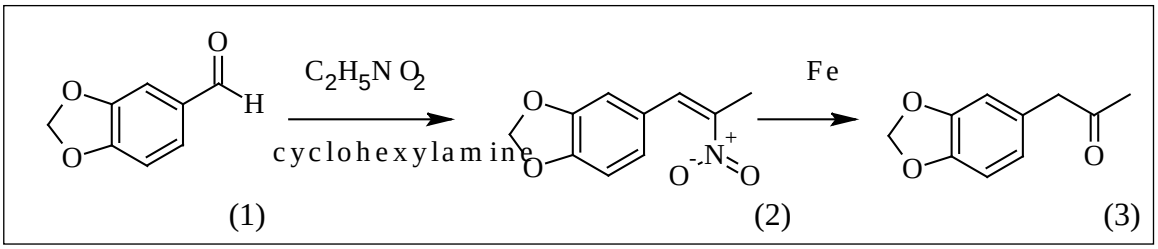


Figure 1.7 3,4-MDP2P synthesis by 3,4-MDP-2-nitropropene (Note: (1) piperonal, (2) 3,4-MDP-2-nitropropene and (3) 3,4-MDP2P) [118].

Another route used to produce 3,4-MDP2P was the Wacker oxidation of safrole using p-benzoquinone, palladium chloride and methanol [120]. More, recently 3-[3′4′(methylenedioxy)phenyl]-2-methyl glycidate, a non-controlled chemical which is made from piperonal, has been used as a precursor to produce 3,4-MDP2P [37, 115].

Unlike other illegal drugs, such as cocaine and heroin, whose production is limited by geography and climate, MDMA can be produced almost anywhere [36]. Production is dependent upon chemical and technological knowledge; the availability of chemicals and equipment; and easy access to literature on the subject, especially from the internet [121]. The choice of synthetic route is largely determined by the availability of precursors and other reagents. The efficiency with which a given amount of precursor can be converted into MDMA is dependent on the different manufacturing methods; an example is the reductive amination method which gives the highest yield for MDMA.

The most popular method of synthesis of MDMA is reductive amination of 3,4-MDP2P with methylamine, because of the high yield of MDMA. Other synthetic routes include

the Leuckart method via 3,4-MDP2P and the direct bromination of safrole where 3,4-MDP2P is not an intermediate.

1.5.2 Methods of synthesis of MDMA

1.5.2.1 Reductive amination

A diversity of the reductive amination method exists because different reducing agents can be used. However, the two most commonly used methods are the catalytic metal reduction and the so called 'cold method' [122]. The catalytic reduction involves the reductive amination of 3,4-MDP2P with methylamine with hydrogen gas over a platinum oxide (PtO_2) catalyst [123]. In the 'cold method' the sodium borohydride ($NaBH_4$) reacts with 3,4-MDP2P and methylamine at low temperature (-20°C – use of freezers). The process is called 'cold method' because the reaction is exothermic and hence the vessel is cooled in order to control the temperature. In the clandestine laboratories were the 'cold method' is used freezers are always present [118] (Figure 1.8).

Other alternative methods of reduction are the dissolving metal reduction, also known as the low pressure reductive amination, using aluminum amalgam (Al/Hg, aluminum foil with mercuric chloride) [124] and sodium cyanoborohydride (NaBH3CN) reduction

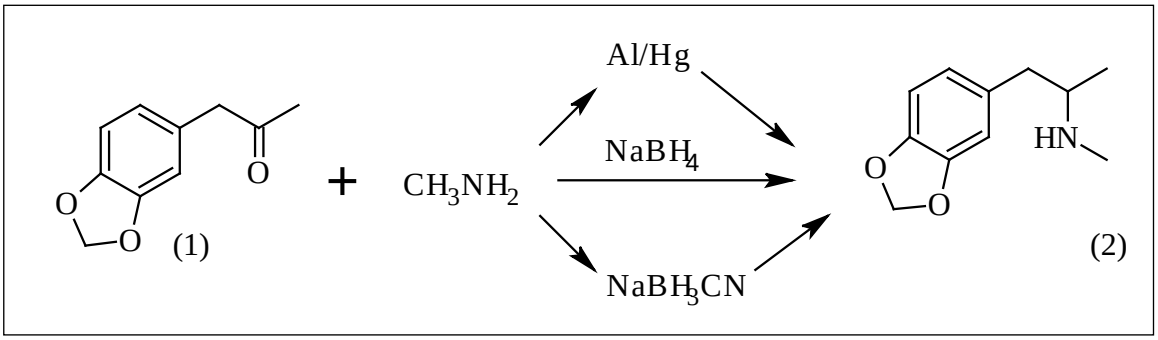


Figure 1.8 Reductive amination methods for MDMA synthesis (Note: (1) is 3,4-MDP2P and (2) is MDMA) [118].

[118]. Contrary to the 'cold method' the sodium cyanoborohydride method is carried out

at room temperature (Figure 1. 8) [12 5]. Substituting methylamine with ethylamine produces MDEA, the use of ammonia gas produces MDA, while dimethylamine produces 3,4-methylenedioxydimethylamphetamine (MDDMA) [12 3], which is a structural isomer of MDEA, a psychoactive substance.

1.5.2.2 Leuckart method

The illicit manufacture of MDMA by the commonly used Leuckart method is analogous to the manufacture of amphetamine by the same method. For MDMA, 3,4-MDP2P and N-methylformamide are reduced using formic acid, producing the intermediate N-formyl-MDMA which is then hydrolised by refluxing with strong acid or base to produce MDMA (Figure 1.9) [123].

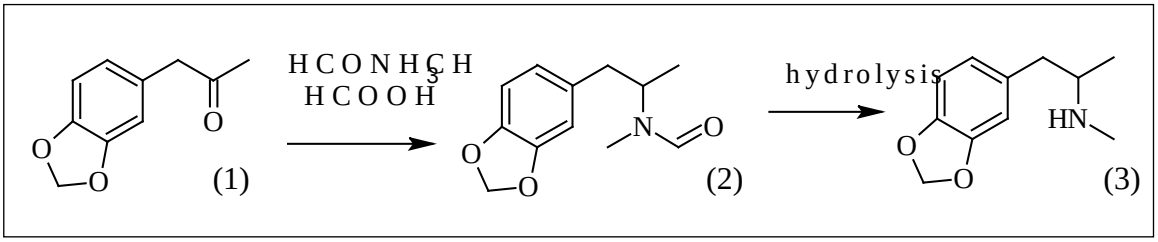


Figure 1.9 Leuckart method for MDMA synthesis (Note: (1) 3,4-MDP2P, (2) *N*-Formyl-MDMA and (3) MDMA) [118].

1.5.2.3 Safrole bromination method

The precursor for the bromination reaction reaction is safrole, which is converted to 2-

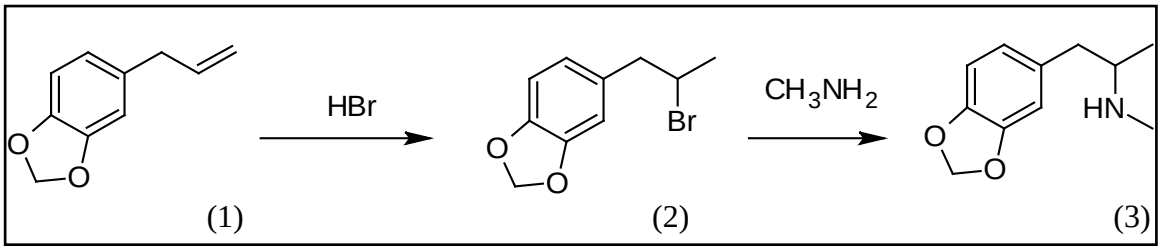


Figure 1.10 MDMA synthesis by safrole bromination followed by methylamine substitution (Note: (1) safrole, (2) bromosafrole and (3) MDMA) [118].

bromosafrole by the addition of hydrobromic acid at low temperature, followed by treatment with methylamine to form the final product (Figure 1.10) [118, 126]. The yield will depend on the water content of the reaction mixture [123]. Other different MDMA-type products such as MDA, MDEA or MDDMA could be produced by the substitution of methylamine [123].

1.5.3 Seizures of precursor chemicals

Between 2006 - 2009 differences were observed in the amount of precursor chemicals seized that could have been used in synthesis of MDMA (Table 1.4). Various factors could have caused the difference in the amount of seized precursor chemicals such as changes in enforcement and changes in manufacture and trafficking [127]. However, the low amount of the precursor chemicals seized considering the amount of 'ecstasy' tablets on the market suggest that much of the precursors used for MDMA synthesis are not detected [36].

Table 1.4 Global amounts of precursor chemicals seized between 2006 and 2009 [36, 127-130].

Precursor Chemicals	Years				To Produce 1 kg of MDMA
	2006	2007	2008	2009	~ Amount of Precursor Needed
Safrole	39 L	45,986 L	1,904 L	1,048 L	1.5 L
Isosafrole	/	225 L	1L	5 L	1.7L
Piperonal	107 g	2 mt	1.4 mt	4.3 mt	2.1 kg
3,4-MDP2P	8,816 L	2,297 L	2,823 L	40 L	1.25 L

In Europe in 2004, there was a decline in the amount (about 17 mt) of reported seizures of 3,4-MDP2P, with the last seizures reported in 2007. The decrease in 3,4-MDP2P seizures could have been caused by tighter controls on the manufacture of this chemical in China (traditionally the source of this chemical), after an agreement between China

and the EU in 2009 to improve precursor controls [36]. However, it was also suggested by the UNODC in 2010 that the decrease in 3,4-MDP2P in Europe could have been caused by an increased demand of this precursor in other parts of the world where MDMA manufactured had increased, such as North America, South-East Asia and Oceania [36].

Those manufacturing MDMA resorted to alternative methods of production and in 2008, 1,900 L of uncontrolled SRO [54], which is typically sourced from South-East Asia, were seized in Europe. Safrole bromination or the production of the precursor chemical 3,4-MDP2P by safrole oxidation are used in the synthesis of MDMA. In 2009 the Government of Cambodia disposed of 15 mt of SRO and seized another 5.2 mt (Table 1.1) [36]. In 2007 45 mt of safrole was seized in Thailand, [36]. Furthermore, in 2010 the first clandestine laboratory for the domestic extraction and processing of SRO for the manufacture of 'ecstasy' was uncovered in Australia [130].

1.5.4 MDMA production

The average yield for MDMA production shows large variation due to a variety of influences including difficulty in obtaining precursor chemicals, differing methods, and levels of expertise of clandestine operators [115, 131]. The manufacture of MDMA, which is slightly more complicated when compared to amphetamine or MA, requires some level of chemical expertise [132] which varies according to the route of synthesis used. MDMA can be produced clandestinely by safrole bromination using safrole oil. However production of the drug is easiest if the starting chemical precursor is 3,4-MDP2P, because MDMA can be made via a simple conversion process using Leuckart method or the reductive amination as described above [133]**.** However, irrespective of variables some methods will be more efficient at converting the precursor to the end-product, MDMA, than others. Information about the manufacturing methods are commonly encountered in clandestine laboratories and from the limited information provided it is clear that average yields show large variation (Table 1.5) [115].

Clandestinely produced MDMA would normally contain impurities which are caused by the manufacturing process. Impurities from carry over precursors chemicals used in the synthesis of MDMA could be detected in the final product such as 3,4-MDP2P, which is the most used precursor chemical in clandestine laboratories in Western Europe [118, 134]. Other impurities emanating from precursor chemicals could include safrole and isosafrole, which both could be used as starting materials to synthesise MDMA [134].

Table 1.5 Average practical yield of MDMA by safrole bromination, Leuckart and reductive amination methods [115].

MDMA Synthesis		
Precursor (1 Kg or 1 L)	**Synthesis Method**	**Average Practical Yield**
Sassafras oil	Safrole bromination	48%
3,4-MDP2P	Leuckart method (with formic acid)	66%
3.4-MDP2P	Reductive amination (Al/Hg)	95%

During the synthesis of MDMA in clandestine laboratories the impurities which could be produced could give information on the synthetic routes used. Impurities such as 3,4-methylenedioxphenyl-2-propanol and N-formyl-MDMA which could be indicative of reductive amination and Leuckart reaction respectively [134, 135]. Impurities could also originate from contact of the finished tablets with materials such a plastic or substances both licit and illicit [135].

In this work MDMA from tablets seized in Malta were analysed for their organic impurities and plausible routes of synthesis of MDMA were determined. Moreover, the total ion chromatogram (TIC) of the organic impurities profiles of the MDMA tablets were used to link or differentiate between the tablets from different batches. As far as is known this is the first study where MDMA-only tablets seized in Malta were analysed for their organic impurities to determine the routes of synthesis of MDMA.

1.6 Production of Compressed Tablets

Once synthesised MDMA is mainly converted to tablets with logo impression, however MDMA could also come in powder (know as “molly” by users) which could me mixed with excipients and adulterants [136].

It is reasonable to assume that producers of ‘ecstasy’ tablets would resort to similar methods used for licit tablet production. This has been corroborated by the discovery of many illicit laboratories in many countries around the world [121, 130] where ‘ecstasy’ tablets were produced and the publication of a catalogue–book by Europol in 2010 of the equipment used at illicit laboratories for ‘ecstasy’ tablet production [137]. However, as far as it is known no research has been done on methods for illicit production of ‘ecstasy’ tablets.

1.6.1 Criteria for licitly produced tablets

The ‘quality’ of tablets can be assessed by five different criteria:

a) ***Uniformity in the dose of the active ingredient(s)*** – In the case of legally produced tablets the amount of medicament is stated and the variation of the active ingredient(s) should vary within accepted limits. This will depend on the mixing of the powders and the uniformity in the mass of the final tablets which will be dependent on the uniformity in the fill of dies;

b) ***Release of the active ingredient(s)*** **–** When the tablet is taken it should readily undergo disintegration so that the prescribed active material is readily available for dissolution, and the release of the active ingredient(s) from the tablets should be controlled and reproducible;

c) ***Friability and hardness (mechanical strength)*** **–** The friability and the mechanical strength, which are closely related, should be enough to withstand abrasion in packing, transportation and handling;

d) ***Appearance, weight and size –*** The tablet should have an elegant appearance and the appearance, weight and size should be consistent;

e) ***Packaging –*** The tablet should be packaged in a safe manner to protect from mechanical shock and from moisture [138].

There is very minimal information in the literature on the manufacture and the quality of 'ecstasy' tablets. This had led part of this study to focus on the above mentioned areas to determine the 'quality' of the illicit tablets. Most of the examined 'ecstasy' tablets were from batches of tablets seized by the Malta Police Drug Squad from traffickers and / or 'ecstasy' tablets sellers. Different batches were normally received at the Malta Forensic Laboratory packed in plastic bags unprotected from moisture.

'Ecstasy' tablets are manufactured in clandestine laboratories by tabletting machines used to manufacture pharmaceutical tablets [139]. Thus it is expected that similar excipients used in the manufacture of licit tablets are used in the manufacture of 'ecstasy' tablets. Hence the plausible identification of the excipient used in the examined 'ecstasy' tablets was also undertaken in this study. The data obtained from the characterisation of the tablets, both physical and chemical, was used to differentiate between or link batches of tablets.

1.6.2 Formulation of tablets

The formulation of tablets, which can be very complex, can vary considerably in ingredients from one manufacturer to another. The powder or granules that are used in tablets are a mixture of the active ingredient(s) and a combination of excipients. The quality of the tablet is dependent on the chemical and the physical properties of active ingredient(s), and on the accurate individual selection and proportions of the suitable excipients. It is usual practice for excipients, which are physiologically inert substances, to be intentionally incorporated into tablets, apart from the active ingredient(s), to play specific functional roles. Almost all tablets contain excipients in amounts normally

greater than the present active ingredient(s) [140]. Excipients which could be organic or inorganic substances are obtained from natural sources, or produced semisynthetically or synthetically [140]. Depending on their functionality in the resultant formulation excipients can be sub-divided into the following classifications:

Diluents These are inert substances, which are known as bulking agents or fillers that are added to active ingredient in sufficient amounts to make a reasonably sized tablet. The size of the tablets is normally kept above 2-3 mm and the mass above 50 mg and so tablets containing low doses of active ingredient(s) would contain a diluent. This agent may not be necessary if the dose of the active ingredient(s) in the tablet is high, thus the range of diluent in the tablets may vary between 5-80%. Diluents when added to tablet formulation enhance the flow, improve cohesion, allow direct compression, and adjust the weight of the tablet according to the die capacity. Lactose, sucrose, sorbitol, starch, powdered cellulose and calcium phosphate are some examples of diluents [126, 141, 142]. Diluents are also expected to be used in 'ecstasy' tablets such as lactose, sorbitol, starch, sodium, and calcium and barium sulphate and phosphate salts [143, 144].

Binders These **s**ubstances, which are added to tablet formulations, provide the necessary bonding between the powders which when compressed form the tablet. Substances that could be used as binders include gelatine, acacia, polyvinylpyrrolidone, hydroxyl propyl cellulose, pregelatinized starch and hydroxypropyl methyl cellulose among others [126, 141]. Binders are also found in 'ecstasy' tablets such as cellulose [143].

Lubricants These substances, which are normally added in the final mixing step, avoid adhesion between the tablet material, the dies and

punches during tablet compression. Also, these agents make easy the ejection of tablets from the die. Some common lubricants include magnesium and calcium stearates, stearic acid and talc [141]. Lubricants, such as magnesium, zinc, aluminium and lithium stearates and boric acid, have also been found in 'ecstasy' tablets [144].

Glidants These are substances which are added in the dry state before compression to improve the flow characteristics of the powder mixture. Common glidants, which are uses in a concentration of less than 1%, include silicone dioxide and talc, which may also serve as lubricant [141].

Disintegrators Substances, insoluble in water at low temperatures, which are added to a tablet to help with its disintegration by swelling. The most popular disintegrants are corn and potato starch. Others include polyvinylpyrrolidone, sodium carboxymethylcellulose, agar and bentonite are also used [126, 141].

Colouring agents Colours are used in compressed tablets to improve the appearance of the dosage form. Most commonly used colourants are synthetic dyes or lakes. Lakes are choice for sugar or film coating as they give reproducible results [141]. The colouring agents found in 'ecstasy' tablets included E 127 (Erythrosine, red dye), E172 (iron oxides and iron hydroxides, yellow dye) and E 141 (copper complexes of chlorophyll, green dye) [144]. In this study colour was used to differentiate between batches of tablets and reflectance spectroscopy was employed to measure the colour of some samples of 'ecstasy' tablets. Moreover, to investigate the possible use of colour of 'ecstasy' tablets as a discriminatory physical feature a photostability study was conducted on samples of tablets

to determine how UV-visible (VIS) radiation may alter the colour of the tablets.

1.6.3 Tablets manufacturing methods

To produce tablets formulators can choose from a range of manufacturing methods including wet granulation, direct compression and dry granulation.

1.6.3.1 Wet granulation

The wet granulation of powders is done to ameliorate the flow and compressibility of the tablet mix [145]. It is a process that uses a liquid binder or adhesive to the powder mix. The binder used would increase the particle strength, and the particle size is optimized for flow. Furthermore, due to improved handling dustiness is decreased, the tablet can be dried to low final moisture content and segregation of fines can be prevented [146]. In general this method produces spherical uniform–sized particles with hydrophobic surfaces with a uniform distribution of the active ingredient. This process exposes liable drugs to moisture and heat conditions, such as acetylsalicylic acid [147], and there is also some material loss during this process [146]. However, stabilizing agents, such as pH modifiers, to stabilize the drug could be used during this process [148]. Though this method is popular because any drug can be wet granulated successfully [145], it has the disadvantage of using a large number of steps making the process more expensive and more complex than other methods [146]. This method, which involves nine steps, includes the blending of the dry powder, drying moist granulation and granule sizing, mixing screened granules with lubricant and tablet compression [145, 146].

1.6.3.2 Direct compression

By contrast with wet granulation, direct compression, which consists of three steps involving the milling of drugs and excipients, the mixing of ingredients and tablet compression, is rather simple and cheap [146, 149]. In direct compression the active

ingredient is mixed with the excipient and lubricant followed by compression in a press to make a tablet without any of the ingredients having to be changed [146, 150]. The method, which makes it easy for the tablet to be processed, would save labour, time, equipment and space [145, 149]. This process, which is ideal for simple formulations, improves the stability of the tablet because neither heat nor moisture is required [146]. However, high-dose (> 50 mg dose [151]) drugs that have poor compression and flow properties, such as certain polymorphic forms of the analgesic and antipyretic acetaminophen [152], and low-dose (≥ 10 mg dose [151]) drugs due to the non-homogenous distribution in the final tablet would not be suitable for this type of manufacturing method [146]. Also drugs with extremely low bulk density, such as the anticonvulsant rufinamide [153], would be difficult to directly compress due to air entrapment, using this method [145].

1.6.3.3 Dry granulation

Dry granulation, which is considered to be a pre-compression process, is used when the tablet ingredients are labile to moisture and heat [146, 154]. Compared to wet granulation this process, which provides added flexibility of dwell time and compression force, provides better stability and thus it could also be used when direct compression is not possible [146]. This method, that is suitable for medium and high doses, improves the flow property of particles by increasing their size and also increases the density of low-density drugs [145, 155]. The elastic recovery of some compounds is decreased by this method thus increasing the final compactability [146]. Dry granulation, which is a six step process, includes the milling of drugs and excipients, mixing, slugging (which are large tablets), dry screening, lubrication and tablet compression [146, 154].

1.6.3.4 The compression of granules or powders

When the mixture is prepared the tablets are formed by feeding the granules or powder in the tablet press and compressing the material between two punches in a die (Figure 1.11). There is no mention in the literature of the favoured clandestine tabletting process used to

produce ‘ecstasy’ tablets. However, Palhol et al [134] suggested that the presence of both palmitic and stearic acid in ‘ecstasy’ tablets indicated that the tabletting process used was “direct compression”.

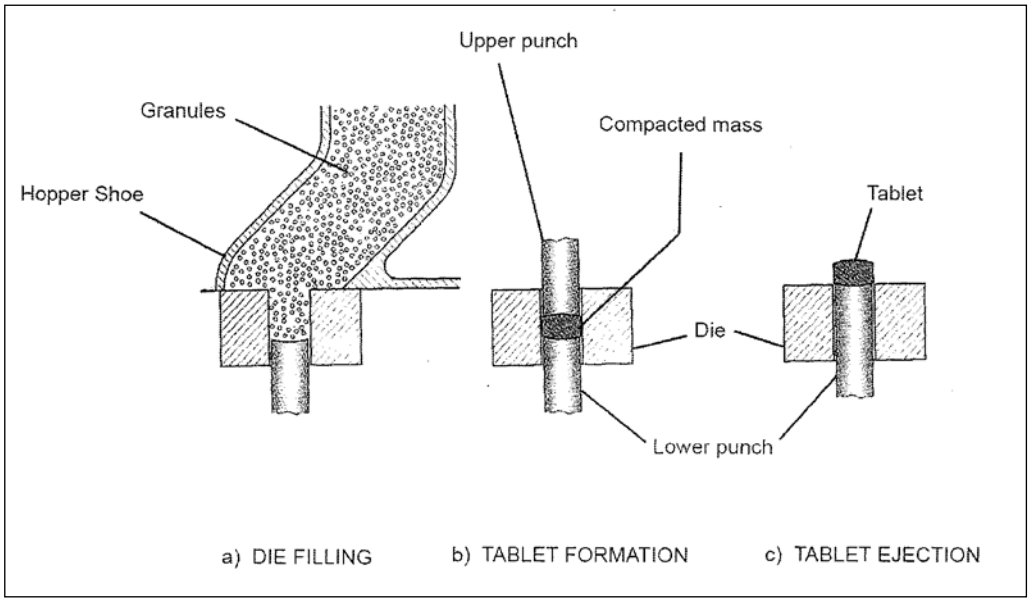


Figure 1.11 Schematic diagram of tablet production [156].

1.6.4 Tablets compressing machines

Compressing machines, also called tabletting machines, range from small inexpensive models that make one tablet at a time, the so called single punch tablet press, with only around a half-ton pressure, to large computerized industrial models, the rotary multiple punch tablet press, that can make hundreds of thousands to millions of tablets an hour with much greater pressure. Both these type of compressing machines have been used for tabletting ‘ecstasy’ tablets [137].

The single punch tablet press, which normally operates at a speed from 1 to 60 tablets per minute, has one station of tooling. These machines normally generate considerable vibrations which could cause segregation of granules and powders in the hopper [157]. Earlier machines had an output between 500 and 1000 tablets per minute with today

presses having output orders of magnitude greater than this [157]. The high-speed machines consist of “double-rotary” presses where the cycle of operation is repeated twice in one revolution of the turret carrying the tooling producing about 11,000 tablets per minute. Most of the above mentioned types of tabletting machines were seized from illegal laboratories producing ‘ecstasy’ tablets [137].

The synthesis and tabletting of MDMA was previously centralized in one laboratory, however nowadays it is less likely that the two processes happens in one laboratory, but tabletting is normally done in other laboratories specializing in production of ecstasy tablets [122].

1.6.5 Defects on tablets

It has been suggested by some that ‘ecstasy’ tablets produced in illicit laboratories will have some form of defects caused by the die [144]. Defects detected in pharmaceutical tablets were also caused by the low quality of raw materials used, the tablet formulation, the poor flow of the powdered mixture, lack of compressibility of the mixture and the tabletting machines [158]. Moreover, other detected defects in pharmaceutical tablets were caused by the milling process (produces fine and constant particle size) which could produce fines causing capping, black spots, laminations and hardness variations [158]. While, as far as we know, there has never been any report or study concerning ‘ecstasy’ tablets manufacture with relation to their physical defects, there have been some mention of ‘ecstasy’ tablets defects, such as weight variation, hardness problems, sticking, capping, chipping and mottling [126, 159, 160], when describing the illicit tablets or writing about ‘ecstasy’ tablet manufacture.

Weight variation of tablets could be caused by a number of factors, such as inadequate lubricant, unsuitable glidant, irregular mixing of lubricants and incorrect tool setting [161, 162], among others. Uneven lengths of the lower punches will result in variations of powder / granular fill in the die which will affect the weight, while variations in the upper punch will effect the compression [161]. According to Tousey [158] controlling

the tablet weight, which is dependent on the space between lower and upper punch, is always the key for hardness control [158]. Hardness variation and insufficiency could be caused by excessive lubricating agents; overly dry mixture and low pressure during tablet manufacture [126].

Another tablet defect that has been described is sticking, which is when the powder sticks to the punches faces [158], which is normally caused by excessive moisture, lack of lubricating agents [163], low melting point substances [162], or worn punches and dies [158]. An additional defect similar to sticking is 'picking', which is caused when the tablet material from the surface of the tablet sticks within the engraving, or debossing on the punch [114, 158]. This is normally caused by insufficient lubricant [163], improper mixing of the total powder or by unpolished dies [163], causing print defects, which are normally seen in 'ecstasy' tablet logos.

Capping of tablets, which is a very common defect, is the complete or partial breaking away of the tablet upper or lower surface as it is ejected [114]. It could be caused by poor blending, the introduction of air, break granules or over blend powder, [163] speedy machines and the strength of the compression [162]. In addition, tablets having sharp edges tend to chip, mostly by rough handling, around the tablet surface where small pieces are broken [114, 162]. Also mottling which is the unequal distribution of colour on the tablet surface, caused by the variation in the colour ingredients, the drug and excipients [114].

On internet website 'Pharmainfo' it is noted that pharmaceutical tablet defects, which could also be observed on 'ecstasy' tablets, could include lamination (similar to capping except that the tablet is separated into layers [114]), which could be caused by small amount of binding agent used [162]. However, this defect, which is often blamed on over compressing [158], is caused by air trapping between the layers of the tablet and this could occur quickly after the compress or during storage [126, 162]. Black spots, which could sometimes be seen on colored 'ecstasy' tablets, could be caused by the inappropriate method of cleaning of the punches and / or the incompatibility between the

excipients and / or active ingredient/s used [162]. Some other defects would include the production of soft tablets, which could be caused by the lack of drying of the mixture used, the low compaction pressure, and the inadequate storage [162], the double impression caused by the uncontrolled movement of the punches with engraving (logos) on them [114] and the unsuitable disintegrating time, caused by the hydrophobic excipients and / or unsuitable disintegrators used [126]. In this research study the defects on the examined 'ecstasy' tablets were noted.

1.7 Legislative Controls

In the UK the potential for abuse of ring-substituted phenethylamines, such as MDMA, had been well anticipated. The generic controls of 1977, in the Misuse of Drugs Act, 1971, in Part 1 of Schedule 2, paragraph (c) define phenethylamines as "any compound (not being methoxyphenamine or a compound for the time being specified in sub-paragraph (a) above) structurally derived from phenethylamine, an *N*-alkylphenethylamine, α-methylphenethylamine, an *N*-alkyl- α-ethylphenethylamine by substitution in the ring to any extent with alkyl, alkoxy, alkylenedioxy or halide substitutes, whether or not further substituted in the ring by one or more other univalent substituents" (Figure 1.12), [164]. Thus, these controls made all compounds that could be derived structurally from phenethylamines Class A drugs under the 1971 Misuse of Drugs Act. This made MDMA then virtually unknown, and not yet scheduled in any UN Convention, illegal in the UK under the Misuse of Drugs Act [164].

In 1986 the World Health Organization (WHO) and the UN Commission on Narcotic Drugs placed MDMA in Schedule 1 of the 1971 Convention on Psychotropic Substances [20]. At that time in 1986 MDMA, which was already under national control in Canada and the UK, was controlled in the US [165]. Although in the US in January of 1988 MDMA was removed from the schedule for controlled drugs it was again scheduled in March of that year [20].

As a state party to the 1971 UN Convention on Psychotropic Substances Malta had an

international treaty obligation to implement and control the drug MDMA. In Malta the substance, which was considered a restricted drug, was first scheduled in 1988 [166]. By

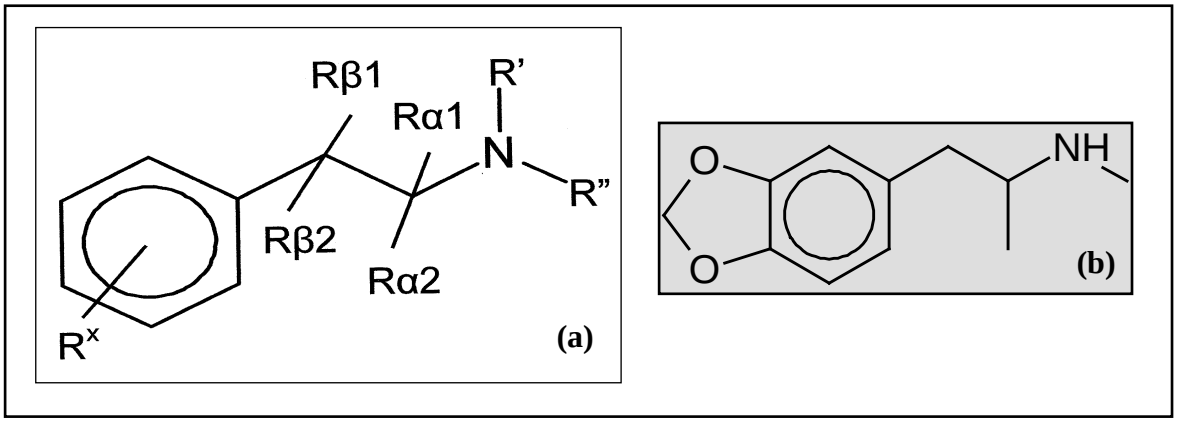


Figure 1.12 (a) The UK Misuse of Drugs Act, 1971, defines phenethylamines as any compound structurally derived from phenethylamine by substitutions on the molecule – R' = H or alkyl; R" = Rα1 = Rβ2 =H; Rβ2 = H, methyl or ethyl; R^x = alkyl, alkoxy, alkylenedioxy or halogen (either singly or in any combination) with or without any other substituents in the ring [164], (b) MDMA chemical structure.

1990 in Malta it had become a criminal offence if one was caught trafficking, dealing or using MDMA [167] and by 1998 the laws for trafficking in psychotropic drugs (under which MDMA is classified) were made harsher, to life imprisonment [168]. Today, under a UN agreement, MDMA is controlled in most countries in the world, and its possession, manufacture, or sale may result in criminal prosecution [169].

Chemicals or substances that are used in the manufacture of other substances are called precursors. Many legitimate industrial chemicals are used in the manufacture of illicit drugs. Since MDMA is a synthetic drug, it is manufactured from chemicals that often get diverted from legitimate businesses. Because precursor chemicals are normally needed to synthesise MDMA governments have adopted legal control as part of their overall drug control and enforcement plans. These controls offer a means of attacking illicit drug production and disrupting the process before the drugs have entered the market.

The 1988 UN Convention "Against Illicit Traffic in Narcotic Drugs and Psychotropic Substances" has established control measures on the diversion of the precursor chemicals

which could be used in the production of illegal drugs, including the synthesis of MDMA [170]. The four chemicals, safrole, isosafrole, piperonal and 3,4-MDP2P, used in the synthesis of MDMA were included in the Convention [170]. Over the years most member states have introduced trade controls on these chemicals. These precursor chemicals were controlled in the US in 1989 [171] in the UK during 1990 [172] and in Malta it was 1998 [173].

Governments also tried to eliminate rave parties by means of enforcement and legislations. This agenda was a response to perceptions that the rave scene became corrupted due to drug use, mainly ecstasy. By 1990 in the UK the Government tried to control the rave culture by legislative control [19]. It is believed that this altered the UK rave scene and managed to push raves parties and the use of ecstasy into the legitimate clubs [19, 174] to avoid prosecution. This was not unique to the UK, the same was happening in the US where many communities and US legislators attempted to reduce the number of rave parties which were considered as locations of ecstasy use [175]. The same happened in Malta when in 1999 the Government tried to control rave parties by legislative control [176]. Nevertheless Townsend, from the Guardian [177] confirmed the continuation of these events in London. Thus raves and the use of illegal drugs during these parties will probably thrive during the coming years.

1.8 Characterisation of MDMA / Ecstasy Tablets

A recurring theme in illicit drug intelligence is to determine the sources of supply and manufacture of MDMA by means of indicative chemical or physical properties. With this aim in mind, the Commission on Narcotics Drugs, in its resolution 1 (xxxix) of the 24 April 1996, requested the Executive Director of the United Nations International Drug Control Programme (UNDCP) to develop standard methods for the profiling / signature analysis of key narcotic drugs and psychotropic substances. Since then, activities initiated by the Scientific Section of UNDCP have been aimed at developing methods for the characterisation and impurity profiling of such substances [178]. A similar project, the “Collaborative Harmonisation Methods for Profiling of Amphetamine Type

Stimulants" (CHAMP), which focused on MA and MDMA, was launched in 2004 and funded by the European Commission [179].

The CHAMP project has shown that data from batch specific organic impurities, such as 3,4-MDP2P and N-formyl-MDMA [179], recorded by a harmonised gas chromatography-mass spectrometry (GC-MS) method and the physical features diameter, thickness, weight and score were found to be reliable and relevant features for forensic drug intelligence perspective. This study also demonstrated the possibility to discriminate / link MDMA samples and batches of tablets, manufactured by a tabletting machine with given settings, using the data from both the organic impurities and the physical features by statistical methods [179]. Thus, physical and chemical characterisation of 'ecstasy' tablets, that could provide information on links between users and suppliers, drug supply patterns and source, could be defined as the profiling of visual (e.g. shape and colour), physical (e.g. mass, diameter and thickness) and chemical features (e.g. chemical impurities arising from precursors and reaction by-products) to provide information for both evidential and drug forensic intelligence purposes [180]. These characteristics occur at different stages during the clandestine production of tablets [181]. The three main steps in the production of ecstasy tablets are:

i. The synthesis of MDMA;

ii. Mixing of MDMA with excipients / cutting agents produce the tablets chemical characteristics;

iii. The manufacture of the tablets by the tabletting machine with given settings which produce the tablets physical characteristics [181].

Generally, the procedure followed by forensic scientists and researchers to characterise 'ecstasy' tablets is to start with examination of the physical features which would include the visual inspection of the features of the seized tablets, such as colour, size, shape and logo if present, and the measurable characteristics, such as mass, diameter and thickness, which could provide important information on the source of the illicit tablets [144].

Nevertheless, similarities between the physical features of different tablets only suggest a relationship at the tableting level, and that the powders used for the tablets could be from different sources [180]. Conversely tablets with different physical features could have been manufactured from the same powder [180]. Thus physical characterisation will be followed by determination of the chemical features, which would normally include the identification of the psychoactive substance/s, adulterants (e.g. ephedrine, caffeine and ketamine [122]) and excipients (e.g. the diluents lactose and sorbitol [122, 144] used to fill up the volume of tablets), which are used in the tablet manufacture and characteristic impurities, such as starting materials, intermediates and by products [144, 182]. In these circumstances a random sample of tablets, normally based on visual similarities would be taken for characterisation. It is generally assumed that batches of 'ecstasy' tablets having similar physical and chemical features come from the same production batch, while tablets with different features are from different batches [139].

1.8.1 Visual and physical characterisation of MDMA / ecstasy tablets

The simplest way of investigating ecstasy tablets is by visually inspecting the physical features of the tablets where the differences and similarities in, for example, the colour and shape of the tablets can be observed and recorded. In the medical field, online visual description of prescription tablets is used to identify a particular tablet, such as the "Pharmer.org" [183] and "Drugs.com" [184] which are websites that help in tablet identification. Another is TICTAC, a UK commercial visual drug database for healthcare and law and order authorities, which contain information on legal and illegal drugs [185].

In forensic science, only a few publications have described results of a detailed physical comparison of tablets and the use of such characterisation from a drug intelligence perspective. However, there have been some attempts to collect visual data of 'ecstasy' tablets, such as the logo project of Europol [186], where a large number of 'ecstasy' tablets logos were collected, and websites such as "ecstasydata.org" [187], where various information on 'ecstasy' tablets, such as logo, active ingredients and percentage

pharmacological content, date when found and country of seizure, were collected. There is little information available in the literature about the use of 'ecstasy' tablet colour, mass, diameter and thickness for intelligence purposes. Some research has focused on the use of microscopes and comparative microscopes to determine tablet shape; to measure the physical features such as tablet width, thickness and angle of breaklines when present; and to compare the damage marks transferred by the punches to illicit tablets [126]. Other researchers such as Zingg [126] and Marquis et al. [181] have focused on similar physical parameters such as logo, breakline, colour, shape, mass, diameter and thickness to establish the physical characteristics of 'ecstasy' tablets. These physical properties were found to be useful for drug intelligence purposes and to be applied to make links or differentiate between tablets of 'ecstasy' tablets [181].

For legally manufactured pharmaceutical tablets, there are pharmacopeial and pharmaceutical criteria for quality control of parameters such as mass, diameter, thickness, hardness, friability and disintegration time of a tablet in water. As far as can be ascertained hardness, friability and disintegration time have not been used to characterise 'ecstasy' tablets. These physical features were investigated in this research study to determine the 'quality' of the 'ecstasy' tablets seized in Malta. These measurable features were also used to differentiate or link batches of tablets.

Drug synthesis and tabletting does not necessary take place in the same location [122]. Differences in the physical features of tablets does not rule out the possibility that the chemical characteristics could be related, because the same manufactured powder may be tabletted by different moulds, different machines and at different tabletting laboratories [180]. Hence a link between samples based on physical features, would only be indicative of the tabletting machine used with a particular settings. Thus, physical characterisation of tablets should be complemented by chemical profiling to get information on the active ingredient/s and excipients used and to possibly determine the synthetic route used.

1.8.2 Chemical characterisation of MDMA / ecstasy tablets

The chemical characterisation, both organic and inorganic, of 'ecstasy' tablets can be divided in two parts: (1) the general profiling where the major and minor substances, such as active substance/s present, the excipients and adulterants (psychoactive substances other than the named substance), are identified and (2) impurity profiling where trace chemicals found in / on the tablet are detected and, where possible, identified. Impurities in tablets can arise from precursors, reaction by-products, synthetic intermediates, impurities from contaminated reagents, all of which can accumulate during the manufacturing process because of inadequate purification methods. Impurities may also arise from packaging, handling and storage [188-190].

Many techniques have been used to determine the chemical composition of 'ecstasy' tablets ranging among others from methods to identify the psychoactive substance/s in 'ecstasy' tablets such as presumptive tests, the most common Marquis test, used to identify drug classes [126], thin-layer chromatography (TLC), a separating technique used for identifying psychoactive substances [126]. Liquid-liquid extraction and solid phase extraction-TLC were also evaluated in a study involving impurities found in an intermediate 1-(3,4-methylenedioxyphenyl)-2-nitropropene in the synthesis of MDMA [191]. In this study liquid-liquid extraction was found to be more effective than solid phase extraction in isolating characteristic impurities from drug matrix [191]. However, in another study the solid phase extraction of impurities in 'ecstasy' tablets was found to be more effective than liquid-liquid extraction when used with GC analyses [188].

The instrumental methods used for chemical characterisation of 'ecstasy' tablets included among others infrared (IR) surface Raman spectroscopy [192, 193] and near IR (NIR) spectroscopy [143]. The Raman spectroscopy was used to differentiate between tablets from different seizures using compositional differences [192]. Studies on 'ecstasy' tablets had shown that distinction between tablets was possible using the difference in excipients, the active drug:excipient ratio and the hydration of the active drug in 'ecstasy' tablets [192, 194]. In another study principal component analysis was applied to the first

derivative of Raman spectra [195]. This method, which was conducted on mixtures of illicit substances with maltose, lactose and flour among others, categorised mixtures of cocaine, heroin and MDMA [195].

Dyes present in illicit tablets were also investigated using a combination of solid-phase extraction, thin layer chromatography (TLC) and capillary zone electrophoresis with diode array detection [196]. The most detected dyes were the hydrosoluble, acidic, synthetic food dyes found in EU countries [196]. It was claimed that the added dye information provided by this method could be useful for comparative purposes and to determine common production sites [190]. In addition NIR spectroscopy, operated in transmittance and diffuse reflectance modes, was developed as calibration model to quantify MDMA and MDEA, in 'ecstasy' tablets [197] and also to study excipients in 'ecstasy' tablets [198]. In another study applying NIR spectroscopy in reflectance mode and high performance liquid chromatography (HPLC), the most common detected 'ecstasy' tablets excipients were lactose, starch, cellulose and sorbitol [143]. The NIR spectra of seized 'ecstasy' tablets containing more than one excipient were more complex and excipient identification was not always possible for some tablets [190].

Other instrumental methods used included X-Ray diffraction; isotopic characterisation [199]; ^{1}H and ^{13}C-nuclear magnetic resonance (NMR) [200]; solid state-NMR (SSNMR) [201]; and chromatographic separation methods such as: HPLC [202]; GC-MS [117, 203-209]; HPLC-MS [210]; and capillary zone electrophoresis. These methods were very effective in providing evidence for the presence of MDMA (or related compounds) within a tablet formulation and as a means to characterise either bulk excipients or manufacturing impurities.

Another research study investigated the identity and percentage composition of the active ingredient(s) in 'ecstasy' tablets confiscated in Taiwan over a three year period [207]. Most (66–71%) of the analysed tablets were found to contain MDMA, as the only active ingredient, which varied from 89 to 133 mg / tablet [207]. While MDMA content in

'ecstasy' tablets in Taiwan decreased over time other compounds were also detected which included caffeine, MA and MDEA among others [207].

Other techniques which are not routinely used and rarely reported include inductively coupled plasma-mass spectrometry, and inductively coupled plasma-atomic emission spectroscopy [144]. These techniques were used to determine trace metal in 'ecstasy' tablets [144, 211]. In a study where inductively coupled plasma-mass spectrometry and inductively coupled plasma-atomic emission spectroscopy techniques were used the stearates of Mg, Zn, Al, and Li were detected [144]. These stearates are used as lubricants in 'ecstasy' tablets manufacture [144]. However, the true potential of the inductively coupled plasma-mass spectrometry and inductively coupled plasma-atomic emission spectroscopy techniques for 'ecstasy' tablets characterisation needs to be further evaluated. Very few studies have been conducted using these techniques possibly because of the lack of quantitative information and variation in the analyte signals due to the poor homogeneity of some illicitly produced tablets [190]. In part of this research study somewhat similar analyses using scanning electron microscopy with energy dispersive X-ray analyser (SEM/EDX) were conducted to determine the elemental ions and their percentage concentration on the surface of 'ecstasy' tablets. Further, the inorganic profiles of the tablets were evaluated for their use to link or differentiate between the tablets.

Considerable research has been carried out on impurity profiling of 'ecstasy' tablets containing MDMA. These tablets are not normally produced in clean rooms as are pharmaceutical products and the chemicals used to synthesise the illicit drug (depending on the route of synthesis used) are not of pharmaceutical quality [122].

When carrying out analyses for intelligence purposes, impurities, which are normally present at low concentrations, need to be isolated from the total tablet content by an extraction process prior to analyses by instrumental methods [188]. The examination of the impurity profiles, including the possible identification of the compounds present, results in an "impurity profile" of the tablet. This can be used to differentiate between, or

to link, different seizures of 'ecstasy' tablets. It would be expected that a "profile" from the same manufacturing batch would have the same impurities in the same relative amounts [134].

Research on impurities found in MDMA synthesised by different methods showed that different synthetic routes and variations to the same synthetic route produced different impurity profiles [190]. When MDMA was synthesised by three different routes: two involving the Leuckart reaction and another described in the Merck patent (safrole bromination) [200] a number of impurities, which were analysed by a combination of: TLC, IR, UV, ^{1}H NMR ^{13}C NMR, XRD, GC and GC-MS, were detected. Identified compounds included precursors safrole and isosafrole; and intermediates isosafrole glycol, 3,4-MDP2P, *N*-formyl-3,4-methylenedioxymethylamphetamine, *N*-formyl-3,4-methylenedioxyamphetamine (for Leuckart Synthesis) and 1-(3,4-methylenedioxyphenyl)-2-bromopropane (MBPBP) (for safrole bromination) [200]. Also, the reaction by-products *N*,*N*-dimethyl-3,4-methylenedioxyamphetamine (DMMDA), a tertiary amine found in illicit MDMA produced by low pressure reductive amination, the methylenedioxy substituted pyrimidines and pyridines analogues were also determined by the same techniques [200].

Impurity profiling was also used to identify the precursors used to produce MDMA [190]. By using GC with flame ionization detection (GC-FID), and corroborating the results by GC-MS, it was possible to confirm that 3,4-MDP2P was the precursor of choice for clandestine MDMA synthesis [190]. GC-FID and GC-MS were also used to identify impurities such as N-formyl-MDMA and 3,4-Methylenedioxyphenyl-2-propanol from Leuckart and reductive amination synthesis respectively [134]. Other impurity profiling analysis, conducted on 'ecstasy' tablets containing MDMA seized in Hong Kong between 2000 - 2001, identified the common precursors 3,4-MDP2P, 3,4-methylenedioxyphenyl-2-propanol, 3,4-methylenedioxy-*N*-methylbenzylamine (MDB), piperonal, and *N*-formyl-3,4-methylenedioxymethylamphetamine [212]. The detection of 3,4-MDP2P as a precursor was attributed to the popular Leuckart and reductive amination syntheses, and MDP to the direct reduction of 3,4-MDP2P [212]. In another research by a different

group, the detection of two major impurities, MDA- and MDMA-dimers, and three substituted pyridines by-products during analysis carried out on MDMA tablets seized in Hong Kong between 2002 - 2004, indicated the use of Leuckart synthesis [209].

Route specific impurities produced during the synthesis of 3,4-MDP2P via isosafrole oxidation and the nitrostyrene route were also identified by GC-MS analysis [135]. In addition, basic and neutral impurities, such as N-methyl-2-methyl-1-(3,4-methylenedioxyphenyl)-ehaneamine and 3,4-methylenedioyphenyl-2-bromopropanme produced during the synthesis of MDMA via Leuckart, bromosafrole and reductive amination routes were also examined [206]. Analyses by TLC, GC and GC-MS carried out on precursors, intermediates, reaction by-products and MDMA, obtained from MDMA synthesis in the laboratory, were used to establish the route of synthesis of the illicit compound by the study of trace impurities [181]. Undoubtedly the above organic impurity profiling methods had helped researchers to identify most of the precursors, intermediates, by products produced during the synthesis of MDMA, thereby generating a "chemical fingerprints" of the differently synthesised MDMA found in different batches of ecstasy tablets.

Over the years legislative controls have been put in place to control MDMA tablets and its precursor chemicals as well as other psychoactive substances used in the production of 'ecstasy' tablets. Thus it was thought that this research study would focus on the physical characterisation and chemical profiling of MDMA / ecstasy tablets to provide information to law enforcement agencies. This study was carried out on 'ecstasy' tablets that were seized in Malta over a five year period from 2006 - 2011.

'Ecstasy' tablets which are illicitly produced are not strictly controlled. Thus the physical features and physical stability of 'ecstasy' tablets were examined using adopted methods from European and British Pharmacopoeia and the International Conference on Harmonisation (ICH). Chemical analyses of 'ecstasy' tablets and chemical profiling of MDMA tablets were carried out using various analytical methods. The data obtained from the physical features together with the chemical profiling was used to try to

discriminate or link batches of 'ecstasy' tablets from same or different seizures. It is hoped that the scientific information gathered during this research study together with the methods used could be of benefit to scientists involved in law enforcement, intelligence gathering that try to determine distribution networks, traffickers of 'ecstasy' tablets and also linking dealer with user of these tablets.

In Malta, where the drug problem is usually limited to the sale and the use of consumer quantities [213], MDMA / ecstasy tablets have also found their place and compared to the use of other illegal drugs these tablets are still popular [47]. However, the research on drug use in recreational settings in Malta is scant. For this reason a field study was carried out to examine the behaviour of party attendees and also to investigate if the substances used, especially 'ecstasy' tablets, were related to the drugs seized by the authorities.

1.9 Rationale for the Thesis

'Ecstasy' tablets could be characterised in terms of a set of selected physical and chemical properties, giving in the process information (i.e. profile) on the particular batch of clandestinely produced tablets. The physical and chemical characterisation of 'ecstasy' tablets was labelled as a "promising methodology" by Swist et al [206] that could provide information on links between users and suppliers, drug supply patterns and source. In this research a set of selected physical (visual and measurable) and chemical features were studied to investigate their use in linking or differentiating between batches of 'ecstasy' tablets seized in Malta over a 5 year period from 2006 – 2011 and the utility of these characteristics for intelligence purposes.

The physical features of 'ecstasy' tablets might have a bearing on whether a scientist feels able to link tablets obtained from different seizures. It is generally assumed that tablets with similar physical features are from the same production batch and were manufactured with the same tabletting machine and punches, while tablets with different

characteristics are from different batches [139]. Unfortunately the physical features of 'ecstasy' tablets for profiling purposes have been neglected in the literature [181].

In this research study to establish the physical characteristics of the tablets in the batches 10 parameters were chosen which included the visual features (logo, breakline, colour, shape), the measurable features (mass diameter, thickness), and the tablet handling characteristics (hardness, friability and disintegration rate). The examinations of the tablets in the batches always started with the description of the visual features. This was always followed by the determination of the measurable features and the tablet handling characteristics of the seized 'ecstasy' tablets, using in the process, for the first time, adopted methods from the European and British pharmacopeia and pharmaceutical criteria.

A gap noted in the scientific literature is the lack of information on the stability of physical features of 'ecstasy' tablets during storage, if the physical characteristics are to be used for drug intelligence purposes. Thus, to address this lack of information three tests were conducted which included a photostability test to investigate possible changes that might occur in the colour, and two tests on the measurable features (mass, diameter and thickness), and tablet handling characteristics (hardness, friability and disintegration rate) of 'ecstasy' tablets at different storage conditions (an accelerated storage experiment - 40°C and 75% relative humidity (RH) for 6 months and a stability experiment - 5, 15, 25, 35 and 40°C and 33 and 75% RH for 16 weeks). Storage conditions were also recommended based on the results obtained.

Some researchers are of the opinion that the physical features of tablets are not considered sufficient enough to provide evidence of a link or to discriminate between seizures of batches of 'ecstasy' tablets [214]. To investigate this issue and to further enhance the discriminative potential of the physical features, chemical profiling, both organic and inorganic, of 'ecstasy' tablets was undertaken. Organic and inorganic profiling was conducted on 'ecstasy' tablets to determine major and minor substances,

such as psychoactive substances and tablet excipients. Impurity profiling of MDMA tablets was also carried out to try and determine the route of synthesis.

In an overview of MDMA-related literature for 2003 on the Multidisciplinary Association for Psychedelic Studies website, it is stated that MDMA used in 'ecstasy' is assumed to be a racemic mixture, and to date, the racemate has been employed in all human trials [215]. While an EMCDDA report on MDMA states that almost all illicit MDMA exist as a racemic mixture [58]. The latest and only study that carried out to determine the enantiomer ratio of MDMA was concluded by the CHAMP Group in 2006 [179]. So it was felt that MDMA tablets seized during the period of this study should be analysed to determine the S-(+)- and R-(-)- steroisomer ratio. The properties of MDMA enantiomers are different the S-(+)- enantiomer being more potent than the R-(-)- enantiomer [73, 80-84, 216, 217]. MDMA has an enantioselective metabolism where the S-(+)- enantiomer is metabolized faster than the R-(-)- enantiomer [65, 88].

Over the period of this study commercial EDM parties in Malta have grown. Scientific literature shows a high level of drug use behaviour by attendees at dance music events [218, 219]. It is also accepted that some illicit drug use would occur amongst the Maltese partygoers who attend EDM parties. However, there is a lack of research on these types of parties that are held in Malta and no information is currently available. To this effect two studies were undertaken to try and investigated the licit and illicit substance use and the pre, during and post party behaviour of partygoers at EDM events in Malta.

1.10 Aims and Hypotheses

The contribution of this project to the scientific literature is three-pronged, involving (a) the physical characterisation and chemical profiling, including the psychoactive substances and enantiomeric ratio of MDMA / ecstasy tablets, (b) using the data obtained to link and discriminate between batches of 'ecstasy' tablets and (c) investigating the licit and illicit substances used at EDM parties in Malta over a six year period and the behaviour of attendees at these events.

1.10.1 Main hypotheses

For this research study five basic hypotheses have been considered which were:

Hypothesis 1: ***The physical state of different batches of 'ecstasy' tablets seized on different occasions in Malta during 2006 – 2011 will be significantly different from each other.***

Hypothesis 2: ***Seized 'ecstasy' tablets will contain MDMA as the predominant psychoactive substance.***

Hypothesis 3: ***The enantiomers ratio of MDMA tablets will be 50:50 in illicit tablets.***

Hypothesis 4: ***The chemical composition of different batches of 'ecstasy' tablets seized on different occasions in Malta during 2006 – 2011 will be significantly different from each other.***

Hypothesis 5: ***Those 'ecstasy' tablets seized by the police at EDM events in Malta will match batches seized on different occasions in Malta.***

Chapter 2

MATERIALS AND METHODOLOGY

2.1 Materials

2.1.1 Solvents and reagents

All solvents and reagents used were of analytical reagent AR grade (Rathburn, Walker-burn and BDH, both UK). The tablets excipients lactose, sorbitol, and starch were purchased from BDH, UK and dibasic calcium phosphate (DCP) from Sigma – Aldrich, Germany. The reference standards MDMA, MDA, amphetamine, BZP, mCPP, caffeine, methandrostenolone, heroin and cocaine, all at a concentration of 1 mg mL^{-1}, tetrahydrocannabinol (THC) at a concentration of 100 μg mL^{-1} and LSD at a concentration of 25 μg mL^{-1}, were purchased from Alltech, USA. The S(+)- and R(-)-MDMA reference standards were generously donated by Dr. Andrew T. Kicman, Drug Control Centre, Department of Forensic Science and Drug Monitoring, King's College London.

2.1.2 Tablets

The examined batches of 'ecstasy' tablets were mostly confiscated at entry points into the country, hidden in false bottoms of suitcases, or found on premises in Malta by the Police Drug. Polythene bags, which are known to be permeable to water vapour, were typically used by traffickers to pack their batches of tablets such that, especially during the summer months, the tablets might have also been subjected to sunlight.

In Malta a police drug case, which could be part of a larger police operation, would normally involve drug seizure(s), which by court order would be handed to the Forensic

Laboratory for analyses. During particular drug cases where 'ecstasy' tablets were involved there have been a number of tablet seizures. In some cases police searches of premises resulted in the finding of 'ecstasy' tablets at different locations. When tablets were found in bags in different locations these were "bagged up" separately and registered as separate seizures even if the tablets in the seizures were visually indistinguishable. Seizures containing visually indistinguishable tablets were characterised by the time and place of the seizure and the total number of 'ecstasy' tablets

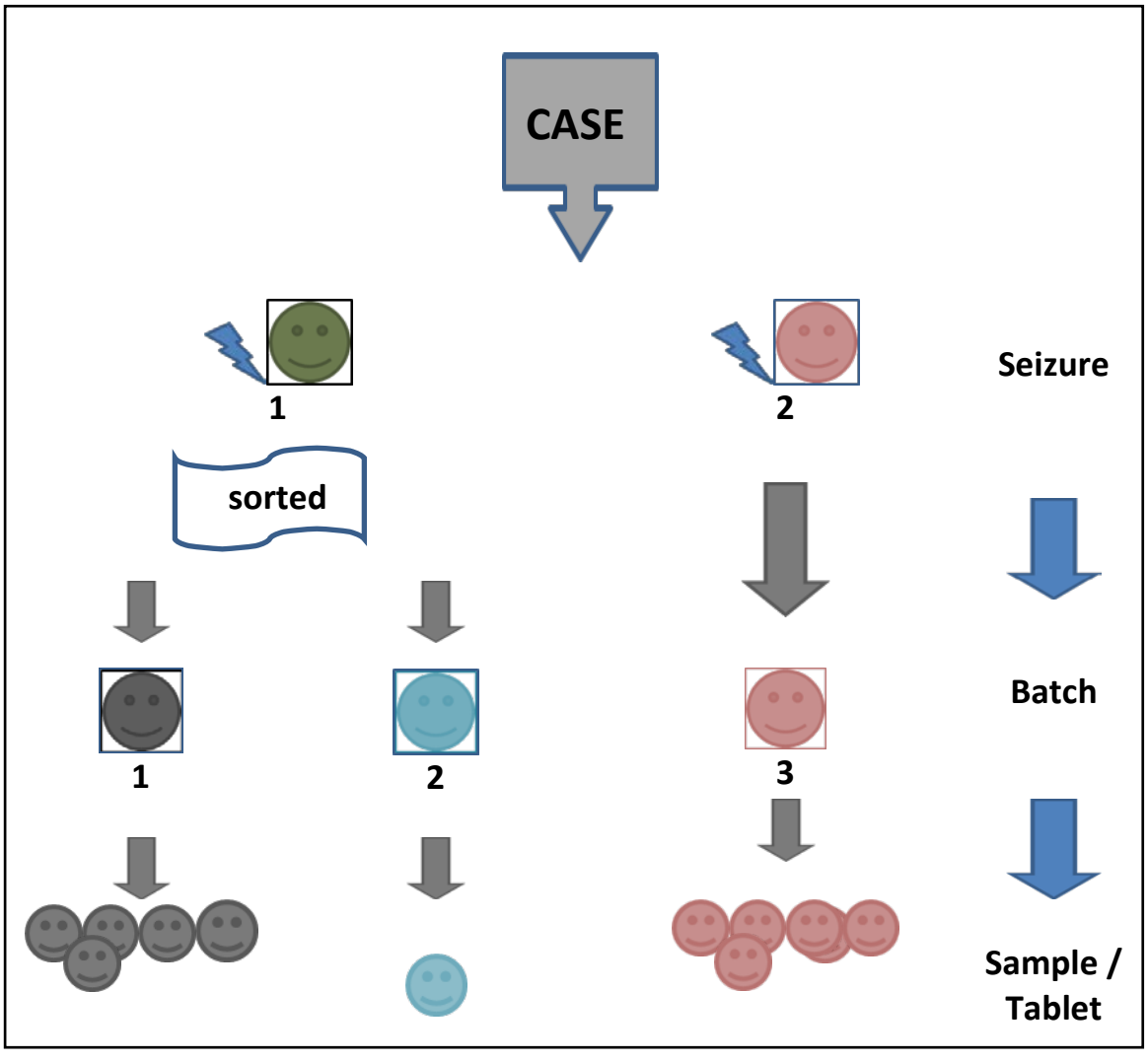


Figure 2.1 Seizures handling of 'ecstasy' tablets for physical and chemical characterisation.

found. Although uncommon, two to four different types of tablets were sometimes found

mixed together in a bag during a seizure. In such cases the analyst at the Forensic Laboratory would sort the tablets according to their visual characteristics. The sorted tablets from the same seizure or the bagged indistinguishable tablets constituted the batch. Random samples of visually similar tablets were used for characterisation and each tablet, being the lowest possible unit of the sample, was visually, physically and chemically examined (Figure 2.1).

2.2 Methodology

Ecstasy tablets from 30 cases of large seizures of tablets (100 or more 'ecstasy' tablets - (EUROPOL criteria for large seizures (E. Mangion, 2006, personal communication Superintendent, Malta Police, Malta's representative at Europol, telephone conversion, 20th November) and 51 small seizures (ranging from 1 to 4 tablets), were investigated over a five and six year period respectively, from 2006 - 2011.

The characterisation of 'ecstasy' tablets is defined in this study as the use of methods to determine the physical features, visual and measurable, and chemical characteristics which included the main active ingredient, enantiomer ratio and impurity profiles of MDMA, major excipients and elemental profiles.

The methods used for physical and chemical characterisation of MDMA / ecstasy tablets are shown in Table 2.1.

The general procedure followed in examining the batches of tablets was first to note the visual features which included the logo, shape, breakline (if present) and colour and then measuring the measurable features. It was expected that tablets manufactured with same tableting machine and punches would have very similar measurable features. Thus the mass, diameter, thickness, hardness, friability and disintegration were measured.

The stability of the physical features of 'ecstasy' tablets, which included colour, mass, diameter, thickness, hardness, friability and disintegration rate were also investigated.

Table 2.1 The investigated characteristics and stability of ecstasy / MDMA tablets.

Visual	*Physical*	*Stability of Physical Characteristics*	*Chemical*
• Logo • Breakline • Colour • Shape	• Mass • Diameter • Thickness • Hardness • Friability • Disintegration rate	• Photostability • Temperature and humidity	• Psychoactive substances and quantity • Percentage isomeric content of MDMA-only tablets • Major excipients • Organic impurity profiles of MDMA-only tablets • Elemenatal profiles

The stability of the physical characteristics was studied to determine any changes that might occur due to different environmental conditions, such as light, temperature and RH, if these features were used for intelligence purposes. In these experiments the colour of tablets was determined by a portable spectrophotometer.

The presumptive colour tests, TLC and GC-MS analyses were used with the scope to help separate tablets in two groups those having MDMA as the main active ingredient and those without MDMA but having other psychoactive substances. All psychoactive substances were confirmed by the use of standard reference samples of different drug compounds, comparing their retention times and mass spectrum obtained from sample analyses.

If tablets were found to contain MDMA their percentage isomeric content was determined. To this end extracts from such tablets had to be derivatised by trifluoroacetic anhydride (TFAA) and analysed by GC-MS using a chiral column.

Tablets found to contain MDMA were further analysed to determine their organic impurity profile to further link or discriminate between the batches. In such cases liquid-

liquid extraction and GC-MS analyses was used. The organic impurity profiles obtained from the tablets was also examined to establish if the synthetic routes used to synthesise MDMA could be determined.

When tablets were found to contain only one psychoactive substance a UV spectrophotometric method for drug quantification was used. The UV method was preferred because it was simple, less time consuming, economical and did not require any elaborate pre-treatment of the drug and tedious extraction procedures usually associated with chromatographic method [220].

To further chemically characterise the batches, tablets were analysed for the presence of major excipients using Fourier transform infrared transmittance spectroscopy (FTIR). It was thought possible that a rapid indication of the major excipient by FTIR directly from powdered tablet was possible.

In final analyses the elemental profiling of 'ecstasy' tablets was conducted using SEM/EDX. The SEM/EDX was chosen because of its low detection limit, 1000 ppm, when compared to both inductively coupled plasma atomic emission spectrometry or inductively coupled plasma mass spectrometry, with a detection limit ranging from 2 to 0.005 ppm and 0.038 to 0.0002 ppm respectively [144]. Thus the idea of using the SEM/EDX for the inorganic analyses of 'ecstasy' tablets was that of detecting elements from the tablets and not elements from external contamination, such as Pb, Ni, Co, which are at very low ppm concentration (0.2 – 0.003 ppm [144]).

The physical and chemical characterisation of 'ecstasy' tablets was done because it was expected that both the physical state and the chemical composition of the batches seized on different occasions would be significantly different (hypothesis 1 and 4 respectively). Also, it was predicted that MDMA would be the predominant psychoactive substance found in the seized 'ecstasy' tablets (hypothesis 2).

2.2.1 Physical characterisation

The visual and physical characterisations of 'ecstasy' tablets have been investigated to determine the potential of such information for forensic drug intelligence purposes. The data from the physical characterisation was used to try to differentiate or link batches of 'ecstasy' tablets from the same or different seizures. The minimal characterisation needed to differentiate or link batches with a reasonable degree of certainty was also determined. The data from the physical characterisation of 'ecstasy' tablets was also examined to determine the information this provided on the illicit tablets seized in Malta. An overview of the investigated physical characteristics of 'ecstasy' tablets and their stability are listed in Table 2.1. The data obtained from the physical characterisation was also used to determine the physical qualities of the 'ecstasy' tablets. Pharmaceutical and pharmacopeial criteria were used as benchmarks to evaluate the qualities of the seized illicit tablets.

2.2.1.1 Visual characteristics of MDMA / ecstasy tablets

Tablets seized as 'ecstasy' tablets (i.e. assumed from their visual appearance to contain MDMA) have the advantage of providing visual information such as logo and breakline, if present, colours and shape.

Logos, breaklines, colours and shapes

For the identification and naming of logos, if present, on tablets the Europol Ecstasy Logo System catalogue, produced by the Drugs Unit of Europol was used [186].

Breaklines on tablets were recorded using the characterisation, (a) no, breakline, (b) yes, breakline and (c) other, type of breakline. The colours and shapes of tablets were also noted.

After the visual characterisation representative samples of the tablets were taken to

determine the measurable physical characteristics, which included the mass, diameter and thickness, and the tablet handling characteristics, which included the hardness, friability, and disintegration. The parameters measured were compared, with limits of tolerance given for pharmaceutical tablets as listed in the European Pharmacopoeia [221].

2.2.1.2 Sampling of tablets

In seizures were a large number of tablets were involved, the number of tablets in the seizure was estimated from the mass of 100 tablets and then working proportionally from the total mass of tablets. A frequentist approach, which assumes that a fixed but unknown proportion of the tablets contained drugs, was used to statistically determine the sample size to be taken for analysis. The hypergeometric sampling method, which is sampling without replacement, was used to take samples from batches of 'ecstasy' tablets having similar visual features [222].

Hypergeometric sampling method

The used hypergeometric sampling method is a statistically-based model involving a defined confidence level with an associated probability of finding failures in a population [222]. The probability that a sample of size n of tablets contains x positives (tablets containing the same active ingredient), given that the population of size N contains N_1 tablets containing the same main active ingredient, can be calculated by:

$$P(X = x|N_1, N, n) = \frac{\binom{N_1}{x}\binom{N - N_1}{n - x}}{\binom{N}{n}}$$

This hypergeometric distribution was used to calculate the sample size n of 'ecstasy'

tablets that had to be analysed such that at least K(= kN) tablets contained the same active ingredient with (1 - α) 100% confidence. For this research work α was taken as 0.05 and k as 0.9 (implying that the required sample size had to guarantee with 95% of confidence that the seizure contained at least a proportion of 0.9 of ecstasy tablets having the same active ingredient). If an assumption is made about the number of tablets expected to contain MDMA, as their main active ingredient, in the sample (usually x), the sample size n can be solved by the above formula. For the cumulative probability $P(X \geq x) = (1 - \alpha)$ and $N_1 = K$. The sample size of 'ecstasy' tablets for analyses was calculated by means of the hypergeometric software provided by ENFSI [223]. If a proportion of 0.9 of the analysed ecstasy tablets, having the same visual features, were found to contain the same active ingredient(s) another sample, using the hypergeometric method, was taken to carry out measurements of the physical features (mass, diameter and thickness).

The "black-box" sampling method was used where the respective batches were put in a black plastic bag and the samples of tablets were taken at random from the plastic bag. In this case this "black-box" sampling method was applied to eliminate (or at least reduce to a minimum) any bias that may be introduced by the person selecting the samples of 'ecstasy' tablets.

2.2.1.3 Physical characteristics of MDMA / ecstasy tablets

Procedures for determining the mass, diameter and thickness

The measurements for the mass, diameter and thickness were done on randomised samples of tablets that were taken from seized different batches of tablets. The tablets were weighed individually using an electronic balance (Sartorius – CP 225D – readability 0.01 mg, linearity 0.03/0.2 mg; precision of measurements $\pm$ 0.01 mg) to determine the individual and the mean mass. The diameter and thickness of the individual tablets were measured by means of a digital caliper (model Mitutoyo – Digimatic series 293 – 0.01mm).

Hardness (mechanical strength)

The test was conducted to determine the force needed to disrupt ecstasy tablets by crushing. This property is controlled in a pharmaceutical environment to ensure that the tablets are firm enough to withstand handling without breaking or crumbling but not so hard that the disintegration time is unduly prolonged.

A sample of 10 tablets was taken at random and subjected to hardness testing using Pharma test – PTB 311E hardness tester. The results were expressed as the minimum, maximum and the mean values of the forces measured, all expressed in N [221].

Friability testing

Friability is a measure of the tendency of a tablet to chip, crack or crumble due to friction and abrasion resulting from movement such as shipping and handling. A friabilator was used (Pharma test – PTF 1DR) to measure friability, by tumbling tablets in a drum. For 'ecstasy' tablets with a unit mass < 650 mg a sample of whole tablets corresponding as near as possible to 6.5 g was taken. The tablets were dedusted prior to testing, weighed and placed in the drum. The drum was rotated 100 times and the tablets were then removed [221], dedusted and weighed again. If the mass loss > 1% the test was repeated twice more on the same tablets and the mean of the three tests determined. A maximum loss of mass (obtained from a single test or from the mean of three tests) of $\leq$ 1% is considered acceptable for most tablets manufactured to industry standards [221].

Disintegration rate

The purpose of testing the rate of disintegration for 'ecstasy' tablets was to determine if these tablets would disintegrate and if so the time taken for this to occur. The disintegration test was carried out using the disintegration apparatus (Pharma test PTZ – Auto 2EZ) which consisted of a basket rack that held 6 plastic tubes, open at the top and bottom. The bottom of each tube was covered by a 10 – mesh sieve. For the

disintegration test six ‘ecstasy’ tablets were selected at random from a batch of tablets and were placed one in each of the tubes of the basket rack. The basket rack was immersed in distilled water, contained in a 1L glass beaker, maintained at 37 $\pm$ 2°C [221, 224]. The time taken by the tablets to disintegrate was recorded. Disintegration was considered to be achieved when no residue, except fragments of undissolved tablet, remained on the screen of the test apparatus. In cases where residue remained on the screen, the residue was checked to confirm that it consisted of a soft mass having no palpably firm, unmoistened core [225].

2.2.2 Stability tests of ‘ecstasy’ tablets

Stability test experiments to determine the effect of environmental conditions, light, temperature and humidity, were conducted on two seizures of ‘ecstasy’ tablets. Two types of stability tests were carried out: accelerated and stress testing. Both were designed to investigate possible physical changes in ‘ecstasy’ tablets. During accelerated test studies pharmaceutical tablets are exposed to stress storage conditions to increase the rate of any possible physical changes [226]. These tests were adopted for use on ‘ecstasy’ tablets in this study.

Photostability of the colours of ‘ecstasy’ tablets was also examined to investigate any potential change in the colour of tablets when these were exposed to visible and UV light. The objective of these experiments was to establish the ideal storage conditions for ‘ecstasy’ tablets so as to minimize changes in the colour characteristic if this feature was to be used for intelligence purposes.

2.2.2.1 Photostability of the colour of ‘ecstasy’ tablets using stress testing

The photostability was adopted from the ICH) method [227]. An actinometer system that was based on quinine monohydrochloride to monitor the light exposure from the near UV spectra region [227] was used.

Photostability experiment of 'ecstasy' tablets

Photostability testing was carried out on 4 different batches of 'ecstasy' tablets: batches 5 and 8 (blue and green tablets with omega logo), batch 11 (white tablets with euro logo), and batch 14 (orange tablets with pisces logo). Forty tablets were taken randomly from each batch and divided in two sub-samples. Each sub-sample was placed in glass petri dish and then one sub-sample covered with aluminium foil. Tablets in the non-covered sub-sample for each batch (exposed sample) were spread in a single layer in petri dishes (Figure 2.2) and positioned so as to provide the maximum area of exposure to the light source which was positioned 20 cm from the tablets. This gave a total of four batches of 'exposed' tablets and four covered.

The samples of 'exposed' and 'covered' tablets were placed together with two actinometric solutions in a quartz spectrophotometer cell (2% aqueous solutions of quinine monohydrochloride dihydrate, with one of the solutions wrapped in aluminum foil, the control solution) in the light chamber (Figure 2.2). The light chamber was illuminated by a cool fluorescent lamp (Sylvania 18 W, 57 V, 1350 lm) for 38 days and 53 minutes to provide a total of 1.2×10^{-6} of lux hours of visible ight and to a UV

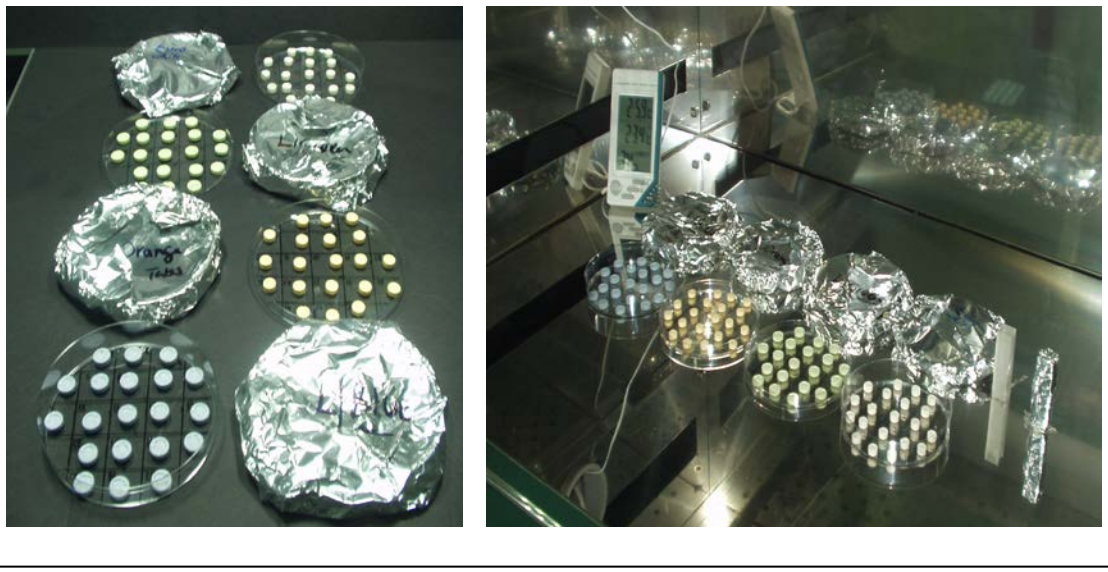


Figure 2.2 Photostability experiment - showing the 8 samples of tablets, 4 exposed samples to light and 4 covered with foil (control samples) in the light chamber.

fluorescent lamp (Sylvania 18 W, 57 V, UVA at 315 – 400 nm – peak at 352 nm and 368 nm) for a total of 11 hours and 7 minutes exposure, to provide a total of 200 Wh m^{-2} of ultraviolet light, according to the ICH adopted method. The chamber was kept at a temperature of 34 $\pm$ 1°C and RH of 29 $\pm$ 1% during the whole experiment. At the end of the exposure period both the exposed and covered samples of tablets were examined for any change in colour.

At the end of the exposure period the colour of both the 'exposed' and 'covered' samples of tablets were measured and the CIELAB coordinates were measured by a portable spectrophotometer (model Micromatch Plus, Sheen Instruments, UK), which was calibrated using three colour standards (Sheen Instruments, UK). The colour standards were the black standard (serial number 188094/6732), the white standard (serial number 188094/6733, D65, L^* 94.56, a^* -13.80, b^* 0.13, ± 0.3) and the green standard (serial number 188094/6734, D65, L^* 79.55, a^* -13.80, b^* 11.54, ± 0.3) respectively.

The measurement principle was based on the measurement of spectral reflectance within the visible spectrum of wavelength from 400-700 nm. The *Commission International de l' Èclairage* (CIE) $L^*a^*b^*$ colour scale system [228] was used. Any change in the colour of the 'exposed' tablets was determined from the difference in the colour measurements between the 'covered' (control) and the 'exposed' samples of tablets from the same batch.

After the exposure period the absorbance of both the exposed (A_T) and non-exposed (Ao) quinine monohydrochloride dihydrate solutions was determined at 400 nm, by means of a UV spectrophotometer (model – Evolution^{TM} 500). The change in absorbance, $\Delta A = A_T - Ao$ was calculated. The length of exposure had to be sufficient to ensure a change in absorbance of at least 0.5 [227].

Calculation of the colour difference

The colour difference (ΔE) between the tablets (control and exposed) after the

photostability stress testing (total 39 days) was calculated using the Euclidean distance between the two points in the colour space (L^*, a^* and b^*), representing them by the formula [229]:

$$\Delta E = [\,(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

The term ΔE was used to describe the colour difference in the CIELAB colour scale system. A change or difference in colour corresponding to a value of $\Delta E > 1.5$ can be perceived by the human eye. The changes in colour, ΔE, of the tablets were compared to published data as shown in Table 2.2 below.

The variance of ΔE ($V\Delta E$) was calculated by the formula:

$$V\Delta E = [((\Delta L^*)^2 / M)V\Delta L^* + ((\Delta a^*)^2 / M)V\Delta a^* + ((\Delta b^*)^2 / M)V\Delta b^*]$$

where $M = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]$

The standard deviation (SD) of ΔE was calculated as the square root of the $V\Delta E$ [230].

Table 2.2 The change in colour, ΔE, of the tablets were evaluated according to visibility of colour difference shown in this table [229].

ΔE	Colour difference
up to 0.2	not visible
0.2 – 0.5	very slight
0.5 – 1.5	slight
1.5 – 3.0	obvious
3.0 – 6.0	very obvious
6.0 – 12.00	large
more than 12.0	

CIELAB method precision and intra-batch variation

The precision was determined by repeatability (intra-day) and intermediate precision (inter-day) on one orange 'ecstasy' tablets with pisces logo (batch 14). Repeatability was evaluated by 6 determinations of the CIELAB coordinates (1 determination was the mean of 3 readings) during the same day, under the same experimental conditions. Intermediate precision was evaluated by 3 determinations during 5 different days. The intra-day and inter-day RSD values obtained by the proposed method were found to be lower than 1%.

The variation (represented by the RSD) for the three coordinates of CIELAB (*L**, *a**, *b**) for the tablets was determined by examining six tablets, taken at random, from a single batch of orange coloured 'ecstasy' tablets with pisces logo (batch 14). The intra-batch variation by the proposed method was found to be lower than 2%. Results are given in Tables 2.3 and 2.4 respectively.

Table 2.3 Intra-day and inter-day precision data for the portable spectrophotometer method for one orange coloured 'ecstasy' tablet (batch 14) for the three coordinates of CIELAB (*L, *a**, *b**).**

Precision	***L****	***a****	***b****
Intra-day (n = 6)	**83.08 (0.04)[a]**	**9.81 (0.51)**	**36.55 (0.25)**
Inter-day - day 1 (n = 3)	83.06	9.81	36.62
Inter-day - day 2 (n = 3)	83.13	9.75	36.51
Inter-day - day 3 (n = 3)	83.11	9.82	36.56
Inter-day - day 4 (n = 3)	83.16	9.67	36.51
Inter-day - day 5 (n = 3)	83.10	9.66	36.53
Inter-day (n = 15) mean	**83.11 (0.05)**	**9.74 (0.82)**	**36.55 (0.14)**

[a] Numbers in parenthesis are the RSDs.

Table 2.4 Intra-batch variation data for the portable spectrophotometer method for 6 orange coloured 'ecstasy' tablets (batch 14) for the three coordinates of CIELAB (*L, *a**, *b**).**

Tablet No.	*L**	*a**	*b**
1	83.03	9.85	36.64
2	83.14	9.74	38.18
3	83.04	9.92	37.22
4	83.63	9.83	36.61
5	83.19	9.76	37.51
6	83.46	9.89	37.44
mean	**83.25 (0.29)[a]**	**9.83 (0.71)**	**37.22 (1.59)**

[a] Nnumbers in parenthesis are the RSDs.

2.2.2.2 Accelerated storage conditions - temperature and humidity

The purpose of accelerated storage conditions testing was to provide evidence on how the physical characteristics of the tablets of 'ecstasy' tablets would vary with time under the influence of temperature and humidity. The accelerated testing, which was adapted from the ICH accelerated storage testing method [231], was carried out on 4 randomly selected samples of 80 'ecstasy' tablets each, from 4 different batches of tablets used for the photostability testing (batches 5, 8, 11 and 14). Eighty bumetanide tablets (Remedica Limited, Cyprus) were used as a control. The bumetanide tablets were chosen because of their similarity in their measurable physical features, mass, diameter and thickness, to 'ecstasy' tablets. The bumetanide control tablets used were not in blister packs but were dispensed from large container packages.

The mass, thickness and diameter of the tablets were measured before the experiment was started. The four samples of 'ecstasy' tablets plus the control bumetanide tablets were stored in a chamber at 40^{o}C $\pm$ 2°C and a RH $\approx$ 75 % $\pm$ 5%. After 3 months about 30 tablets (corresponding as near as possible to 6.5 g) were taken from each of the 4 samples

and from the bumetanide control tablets and the mass, diameter, thickness, friability, hardness and disintegration measured (as described in Section 2.2.1.3). The same procedure was repeated on all the samples and control tablets after 6 months of exposure [231].

2.2.2.3 Effect of temperature and humidity on physical characteristics

A further experiment was conducted to investigate the effect of temperature and humidity on the physical characteristics of 'ecstasy' tablets. The tablets for this experiment were taken from batch 33 (white with versace) which contained 837 tablets.

The method that was used, to create RHs of 33 $\pm$ 1% and 75 $\pm$ 1% respectively, was based on the use of 2 saturated salt solutions (approximately 5% undissolved salt in solution) of magnesium and sodium chloride ($MgCl_2$ and NaCl), respectively. Both salts were of AnalaR grade and deionized water was used for dilution of the salts to create the saturated solution. Each of the saturated salt solutions was put into four separate glass containers, with rubber sealable tops, 10 cm high and 8 cm diameter. About 3 cm of saturated salt solution was placed in each container, leaving 7 cm above the solution to contain air at a fixed humidity. Prior to the start of the experiment the glass containers containing the saturated salt solutions were left for 24 hr to stabilise to the temperature and RH indicated below.

A total of 80 ecstasy tablets, which were taken at random from batch 33, were divided into 8 groups of 10 tablets each for each test condition. The 8 groups of tablets were each put in a plastic net pocket and were suspended above the saturated solution, in each container and kept for a total of 16 weeks under the following conditions:

RH of 33 $\pm$ 1% ($MgCl_2$) - 4 groups - °C				**RH 75 $\pm$ 1% (NaCl) - 4 groups - °C**			
05	**15**	**25**	**35**	**05**	**15**	**25**	**40**

During this period the temperature and RH, which is the amount of water vapour in the

air at a particular temperature, were checked periodically with a calibrated thermohygrometer (Cooper-Atkins, Model SRH77A). The thermohygrometer deviated by no more than $\pm$ 1°C for the five different temperatures 5, 15, 25, 35 and 40°C and $\pm$ 3% for the RH 33 and 75%. Prior to the experiment the tablets has been stored in an air conditioned room at RH of 25% and a temperature of 25 $\pm$ 2°C.

a) ***Baseline measurements:*** The mass, diameter and thickness of all the 80 tablets contained in the 8 samples were measured at the start of the experiment together with hardness. Extra samples were taken to measure the hardness of the tablets;

b) ***Tests carried out during the experiment:*** The mass, diameter and thickness of all the tablets in the 8 samples were measured every week till week 5, and then every 2 weeks from week 8 to week 16. On week 16 the hardness of all the 80 tablets was determined.

2.2.3 Chemical characterisation

To chemically characterise the batches of 'ecstasy' tablet seized over a five year period (2006 – 2011) several different experiments were conducted. The psychoactive substances (if any) in the tablets were detected using routine colour, TLC and GC-MS. The quantification of tablets containing one active ingredient was done by UV spectrophotometry. Moreover, the percentage isomeric content and the organic impurity profiles of MDMA-only tablets were also determined. The major excipients and the elemental profiles of the tablets were also investigated using FTIR transmittance spectroscopy and SEM/EDX analyses (2.1 Table).

2.2.3.1 Experiment A: Detection and quantification of psychoactive substances in batches of 'ecstasy' tablets

Colour tests

Colour tests were used as a first screening procedure to provide an indication of the

presence or absence of drugs in 'ecstasy' tablets. A sample of tablets from each seizure (hypergeometric sampling) was individually crushed to a fine powder using an agate and mortar. Independent colour tests were then conducted on each powdered sample using Marquis, Simons's, Chen's and Scott's reagents [232]. A small amount (1 – 2 mg) of the crushed tablet was placed in a depression on a spot plate and 1 drop of reagent was added. The mixture was mixed by means of a capillary tube and any colour change was noted. Negative and positive controls of amphetamine, MDMA, MDA, BZP, mCPP, caffeine, methandrostenolone and cocaine were also tested with the reagents and any colour change obtained with the tablets was compared with the control mixtures. The colour test reagents were prepared as follows:

- ***Marquis reagent [232]:***

  A 1% solution of formaldehyde (40%, v/v) in concentrated sulphuric acid was prepared.

- ***Simon's reagent [232]:***

  Solution 1 was prepared by dissolving 1g of sodium nitroprusside in 100 mL of water and then 2 mL of acetaldehyde was added to the solution with thorough mixing. Solution 2 was freshly prepared by dissolving 2g of sodium carbonate in 100 mL of water.

- ***Chen's reagent [232]:***

  Solution 1 was prepared by adding 1 mL of glacial acetic acid to 100 mL of water (=1% (v/v) aqueous acetic acid solution). Solution 2 was prepared by dissolving 1 g of copper (II) sulphate in 100 mL of water (=1% (w/v) aqueous copper sulphate ($CuSO_4$) solution). Solution 3 was prepared by dissolving 8 g of sodium hydroxide in 100 mL of water (= 2M aqueous sodium hydroxide solution).

- ***Scott's reagent [232]***

  Solution 1 was prepared by dissolving 2g of cobalt thiocynate in 100 mL of water and then the solution was diluted 1:1 with glycerine. Solution 2 was concentrated hydrochloric acid, while solution 3 was chloroform.

Thin layer chromatography (TLC) of 'ecstasy' tablets

TLC was used as a second test for the presumptive identification of the drug(s) found in the tablets. A known mass of each powdered samples was dissolved in dichloromethane:methanol (1:1; v/v) (≈ 5 mg of sample per mL of solvent). This concentration was based on the premise that ecstasy tablets contained between 30 to 82 mg of MDMA [233] in a matrix of excipients, giving a solution of MDMA approximately 0.3 - 1.0 mg mL^{-1}. Test solutions, positive and negative controls (negative control: dichloromethane:methanol (1:1, v/v); positive control: MDMA at 1 mg mL^{-1}) were developed on a silica gel 60 F_{254}, 0.25 mm pre coated TLC plate (Whatman Ltd. Maidstone, UK) using a mobile phase of methanol:strong (35%) ammonia solution (100:1.5 v/v). A volume of about 5 – 10 μL of sample solution and ≈ 4 μL of positive control were spotted on the TLC plates. Prior to TLC the plate was sprayed with 0.1M potassium hydroxide in methanol and left to dry. Detection was achieved by spraying with acidified iodoplatinate solution (0.25 g of platinic chloride and 5 g of potassium iodide dissolved in 100 mL of water and then 5 mL of hydrochloric acid added to the solution [232]) and and Fast Black K salt (solution A: a 1% solution of Fast Black K salt in water (2,5-Dimethoxy-4-((4-nitrophenyl)azo)benzene diazonium tetrachlorozincate (2:1)) and solution B: a 1 M NaOH [123]).

Confirmation of active substances in tablets by GC-MS

A portion of powdered tablet (20 mg) was shaken with distilled water (2 mL) and to this a solution of 1 M NaOH was added drop-wise to pH 10. The alkaline aqueous solution was extracted with 2 x 5 mL of chloroform then ransferred to the GC-MS for analysis.

Chromatographic and mass spectral data were obtained on a Hewlett Packard 6890 gas chromatograph coupled with a Hewlett Packard 5973 mass selective detector. The GC was fitted with an HP-5 (5% phenyl / 95% methylpolysiloxane) capillary column 30 m x 0.25 mm i.d., 0.25 μm film thickness and helium was used as a carrier gas at a flow rate of 1.0 min^{-1}. The injection (≈ 2 μL) was made in split mode (20:1) with the injection port at 250°C. The GC oven temperature was programmed as follows: 60°C for 2 min, 20°C min^{-1} to 250°C and 15°C min^{-1} to 300°C, with a 6 min final hold time. The mass spectrometer was operated in electron impact ionization mode (EI) and the electron energy was 70 eV. The temperature of the MS source was 230°C, MS quadrupole 150°C and transfer line 280°C. A full scan mass spectrum 40-500 amu was obtained at a scan rate of 0.3 scan s^{-1}.

Analyses for the presence of amphetamine in 'ecstasy' tablets

In order to test for the presence of amphetamine in tablets derivatisation was undertaken using carbon disulphide (CS_2). Powdered tablets (20 mg) were dissolved in 1 mL of ammonical methanol (100 mL of methanol containing 200 μL of 0.880 ammonia) and combined with 2 mL of CS_2 [234]. The mixture was then thoroughly shaken and allowed to stand, at room temperature, for 30 min before being evaporated to dryness under nitrogen and re-suspended in 1 mL of methanol for analysis using GC-MS (EI) as described above.

Determination of mCPP by ^{1}H NMR

The isomers of chlorophenylpiperazine (CPP) identified in 'ecstasy' tablets were determined using ^{1}H NMR because GC-MS could not distinguish the different isomers. A portion of approximately 2 mg of crushed tablet was shaken with 1 mL of deuterated methanol (MeOD). After centrifugation for 1 min at 1000 rpm, 0.6 mL of the supernatant was transferred to a 5 mm NMR tube. ^{1}H NMR spectrum was acquired with a Varian ^{Unity}INOVA 500 MHz spectrometer. 1H chemical shifts were referenced relative to external 4,4-dimethyl-4-silapentane-1-sulfonic acid, the proton calibration standard.

Quantifying drug content in tablets by UV spectrophotometry

A UV spectrophotometer (model Evolution^{TM} 500) was used to determine the quantity of drug content in tablets. A sample of ten tablets was taken from each batch. Tablets were crushed, prepared in solution in methanol at a concentration of 1 mg mL^{-1}, sonicated (10 min) and then centrifuged for 3 min at 2,000 rpm to remove solid material. The tablets solutions were diluted about 10 times by methanol. The maximum absorbance (methanol maxima used: MDMA - λ 285 nm, BZP - λ 258 nm, mCPP – λ 249, caffeine – λ273 nm, methandienone – λ 245 nm and bumetanide – λ 270 nm) values for each tablet solution were recorded within the wavelengths of 200 - 400 nm and the amount of the active ingredient in each tablet was determined against standard solutions. The UV spectra of the psychoactive substances from the tablets were verified by comparing the ratio of peak height to trough depth (peak / trough ratio = θ trough – θ peak / θ trough) of the maximum absorbance peak with that of the respective reference standard.

UV spectrophotometric method validation for the MDMA assay

Specificity

Specificity was evaluated by analysing methanol extracts from mixture of possible excipients without MDMA used in 'ecstasy' tablets. Three mixtures of two excipients

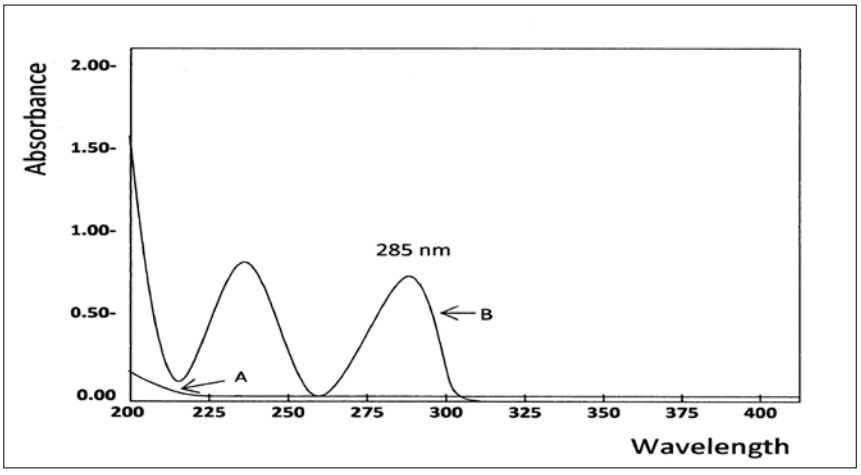


Figure 2.3 UV spectra of (A) in house mixture of excipients (magnesium stearate and lactose) and (B) reference standard of MDMA solution in methanol.

each, all containing magnesium stearate (ratio of 1.5:100, of magnesium stearate) with (a) lactose, (b) sorbitol and (c) starch. The system response was examined for the presence of interference or overlap with MDMA responses at 285 nm. Absorption spectra did not show any possible interference from the excipients at 285 nm (Figure 2.3).

Linearity

The analytical curve as obtained with 5 concentrations of reference solution of MDMA diluted in methanol in the range of 15 - 40 μg mL^{-1} (15, 20, 25, 30, 35 and 40 μg mL^{-1}). Each solution was prepared in triplicate. The linearity was evaluated by linear regression analysis which was used to calculate the correlation coefficient, y-intercept and slope of the regression line.

A linear relationship was found between the absorbance and the concentration of MDMA in the range of 10 to 40 μg mL^{-1} in methanol. The correlation coefficient was 0.9765 indicating excellent linearity. The representative linear equation was y = 0.0236x + 0.1437 (Figure 2.4).

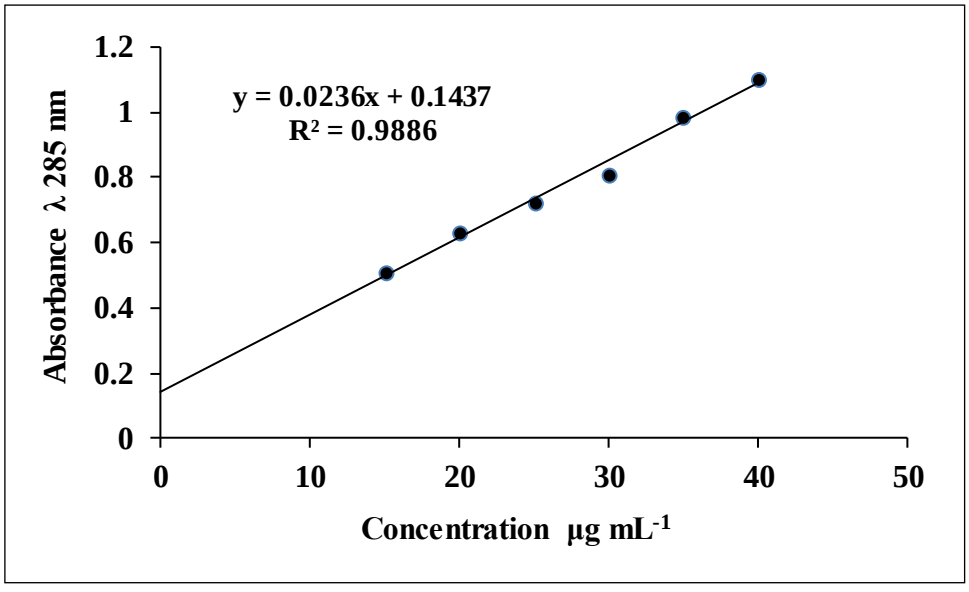


Figure 2.4 The linear relationship between the absorbance and concentration of MDMA in the range of 15 to 40 μg mL^{-1} in methanol.

Precision

The precision was determined by repeatability (intra-day) and intermediate precision (inter-day). Repeatability was evaluated by assaying 6 determinations of the same concentration (30 μg mL^{-1}) of MDMA reference solution diluted in methanol, during the same day, under the same experimental conditions. Intermediate precision was analysed comparing the assays in 3 determinations at the same concentration (30 μg mL^{-1}) during 3 different days. Precision (repeatability and intermediate precision) was expressed as RSD). The intra-day and inter-day RSD values obtained by the proposed method were found to be lower than 1.5 (Table 2.5).

Table 2.5 Intra-day and inter-day precision data of UV spectrophotometric method for MDMA diluted in methanol (30 μg mL^{-1}).

Precision	Mean Absorbance	RSD
Intra-day (n =6)	**0.807**	**0.10**
Inter-day		
Day 1 (n = 3)	0.809	0.37
Day 2 (n = 3)	0.800	1.00
Day 3 (n = 3)	0.799	1.13
Mean (n = 9)	**0.803**	**0.75**

Limit of detection (LoD) and limit of quantification (LoQ)

The *LoD*, is the minimum concentration of MDMA which could still be detected by the analysis method at a specified level of confidence and the *LoQ* is the minimum concentration of MDMA that could still be quantitatively detected with accuracy and acceptable precision by the analysis method. The *LoD* and *LoQ* were determined in the residual standard deviation and regression line and slope. A plot of absorption versus concentration (μg mL^{-1}) was drawn and *LoD* and *LoQ* values were predicted by STEYX

(SD) and slope (S) method using the formula 3.3 x SD / S for *LoD* and 10 x SD / S for LoQ. The calculated values for *LoD* and *LoQ* were 3.71 and 11.23 μg mL^{-1} respectively.

Robustness

Robustness of the proposed method was determined by the analyses of 3 determinations at the same concentration (30 μg mL^{-1}) of MDMA reference solutions diluted in methanol at stored at 3 different temperatures (4, 25 and 35°C). The robustness RSD values were found to be lower than 1.5% (Table 2.6).

Table 2.6 The robustness data of UV spectrophotometric method for MDMA diluted in methanol (30 μg mL^{-1}) at three different temperatures (4, 25 and 35°C).

Robustness	Mean Absorbance	RSD
Temperature °C		
4 (n = 3)	0.799	0.63
25 (n = 3)	0.804	0.50
35 (n = 3)	0.798	1.38
Mean (n = 9)	**0.800**	**0.38**

2.2.3.2 Experiment B: Specific identification and percentage isomeric content of MDMA-only tablets

It is normally assumed that MDMA is formulated in tablets using a racemic mixture (1:1) of its enantiomers. MDMA has an enantioselective metabolism, the (S)-enantiomer being metabolised faster than the (R)-enantiomer. To separate the two enantiomers, MDMA was derivatised using TFAA and the resultant mixture was analysed by GC-MS using an Rt-βDEXcst-TM capillary column. The column had a stationary phase coating of a β-cyclodextrin which was diluted in polysiloxane polymer (Thames Restek UK).

A portion (10 mg) of crushed MDMA tablet was dissolved in 2 mL of absolute ethanol. The mixture was shaken for 10 min on an orbital shaker (Lab-Line, model 3520) and then

filtered using an 'Anotop' 10 (0.2 μm) filter (Whatman) to remove the tablet excipients. To 100 μl of each filtrate, which was dried by a stream of air at 50°C, were added 20 μl of ethyl acetate followed by the addition of 100 μl of TFAA. The derivitisation mixtures were incubated in capped bottles at 70°C for 20 min. Following the incubation samples were evaporated to dryness under a stream of air at 55°C and reconstituted with 100 μl of ethyl acetate. The same method was used for the MDMA standard (1mg mL^{-1}), where a sample of 100 μl of the standard was used and after derivatisation with TFAA was reconstituted with 100 μl of ethyl acetate.

GC-MS analyses was carried out on a Hewlett Packard 6890 GC coupled with a Hewlett Packard 5973 mass selective detector. The GC was fitted with an Rt-βDEXcst-TM capillary column 30m x 0.25 mm i.d., 0.25 μm film thickness (cyclodextrin material added to 14% cyanopropylphenyl – 86% dimethyl polysiloxane). Helium was used as a carrier gas at a flow rate of 1.0 mL min^{-1} (analysis was also carried out at flow rates of 2 and 5 mL min^{-1}). The injection (≈ 1 μL) was made in split mode (35:1) with GC with injection port 200°C and a GC oven temperature isothermal at 160°C. The mass spectrometer was operated in electron ionization mode (EI) with electron energy 70 eV. The temperature of the MS source was 230°C, MS quadrupole 150°C and transfer line 160°C. The MS was operated in full scan mode, scanning 40-500 amu at a scan rate of 0.3 scan s^{-1}. The isomeric content ratios of 22 MDMA tablets were measured using software integration (Agilent Chemstation) [179] (Figures 2.2 and 2.3). Since the peaks were merged the process used by the integrator was that of the dropping perpendicular. The area of the first peak was calculated from the start of the peak till the valley and that of the second peak from the valley until the end of the peak. Then the peak areas were ratioed.

Method validation for derivatised single enantiomer and racemic MDMA

Single MDMA enantiomers (S(+)- and R(-)-) were derivatised using TFAA and analysed by GC-MS on an Rt-βDEXcst-TM capillary column. The two single peaks had retention times of 37.6 min for the S(+)-MDMA and 39.9 min for R(-)-MDMA. The

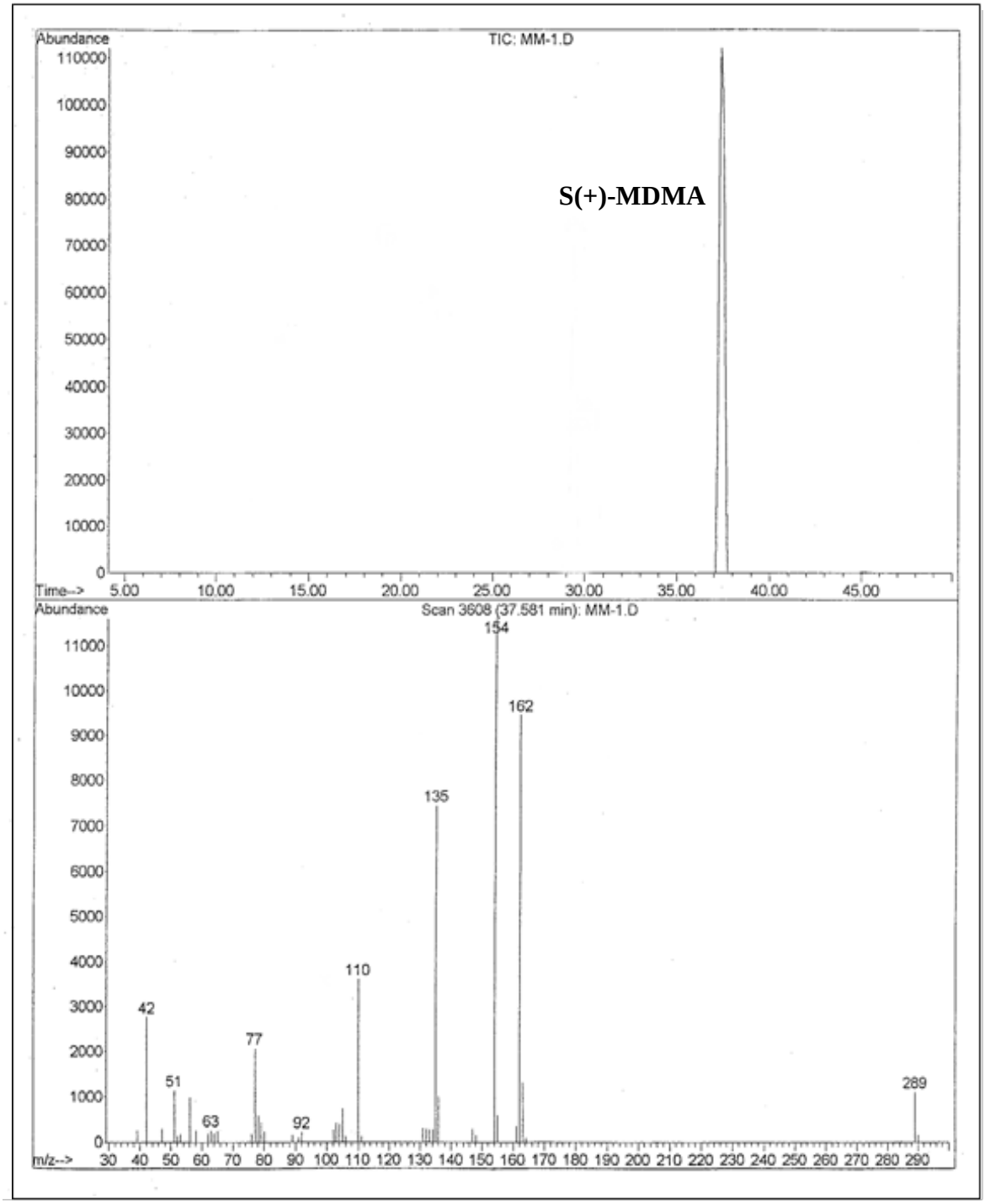


Figure 2.5 The TIC (*Rt* 37.6 min) of the TFAA derivatised S(+)-MDMA control (concentration 1 µg µL^{-1}). The EI mass spectrum of the S(+)-MDMA with base peak at m/z 154, ions at m/z 162, 135, 110 and 42 and molecular ion at m/z 289.

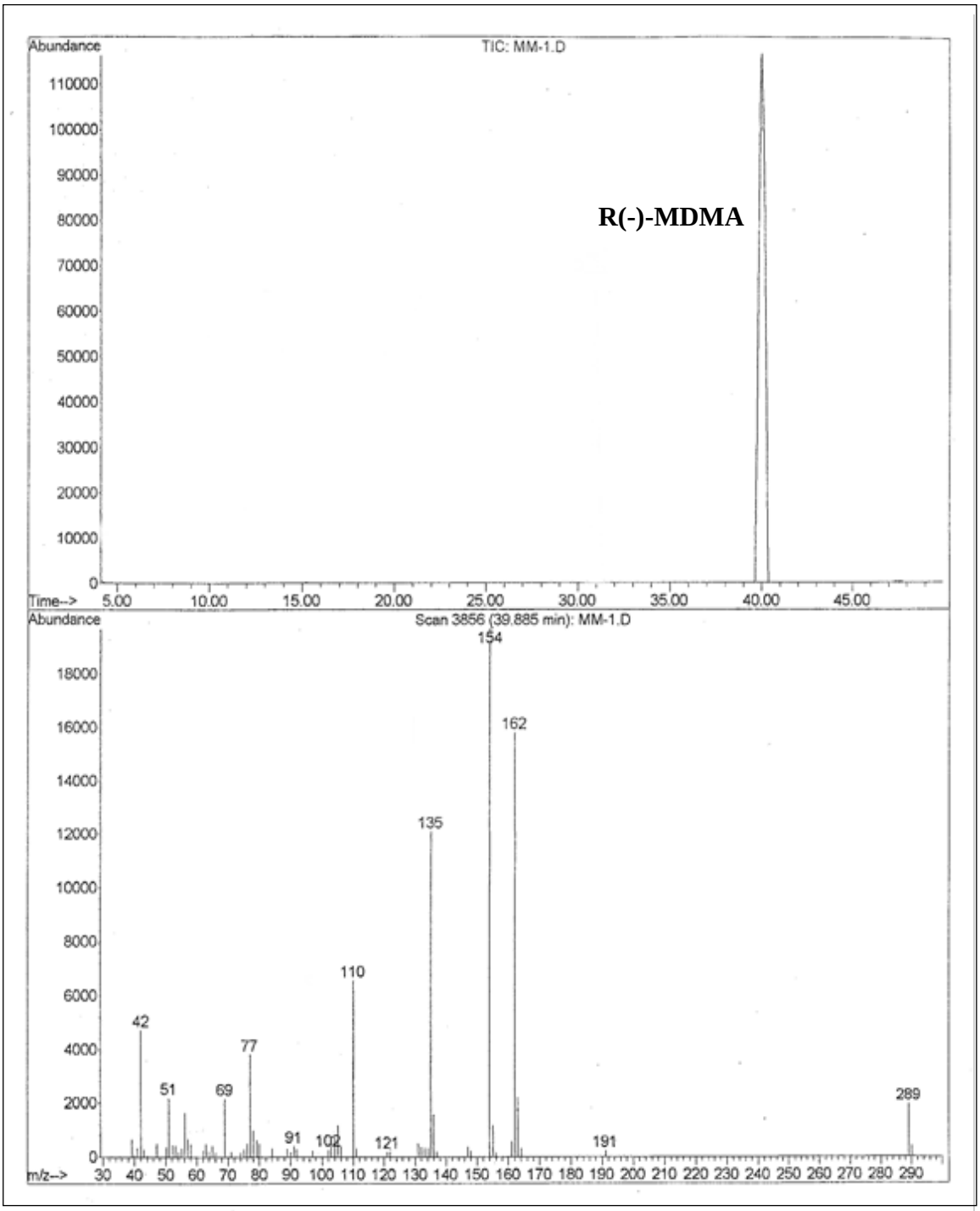


Figure 2.6 The TIC (*Rt* 39.9 min) of the TFAA derivatised R(-)-MDMA control (concentration 1 µg µL^{-1}). The EI mass spectrum of the TFAA - R(-)-MDMA with base peak at m/z 154, ions at m/z 162, 135, 110 and 42 and molecular ion at m/z 289.

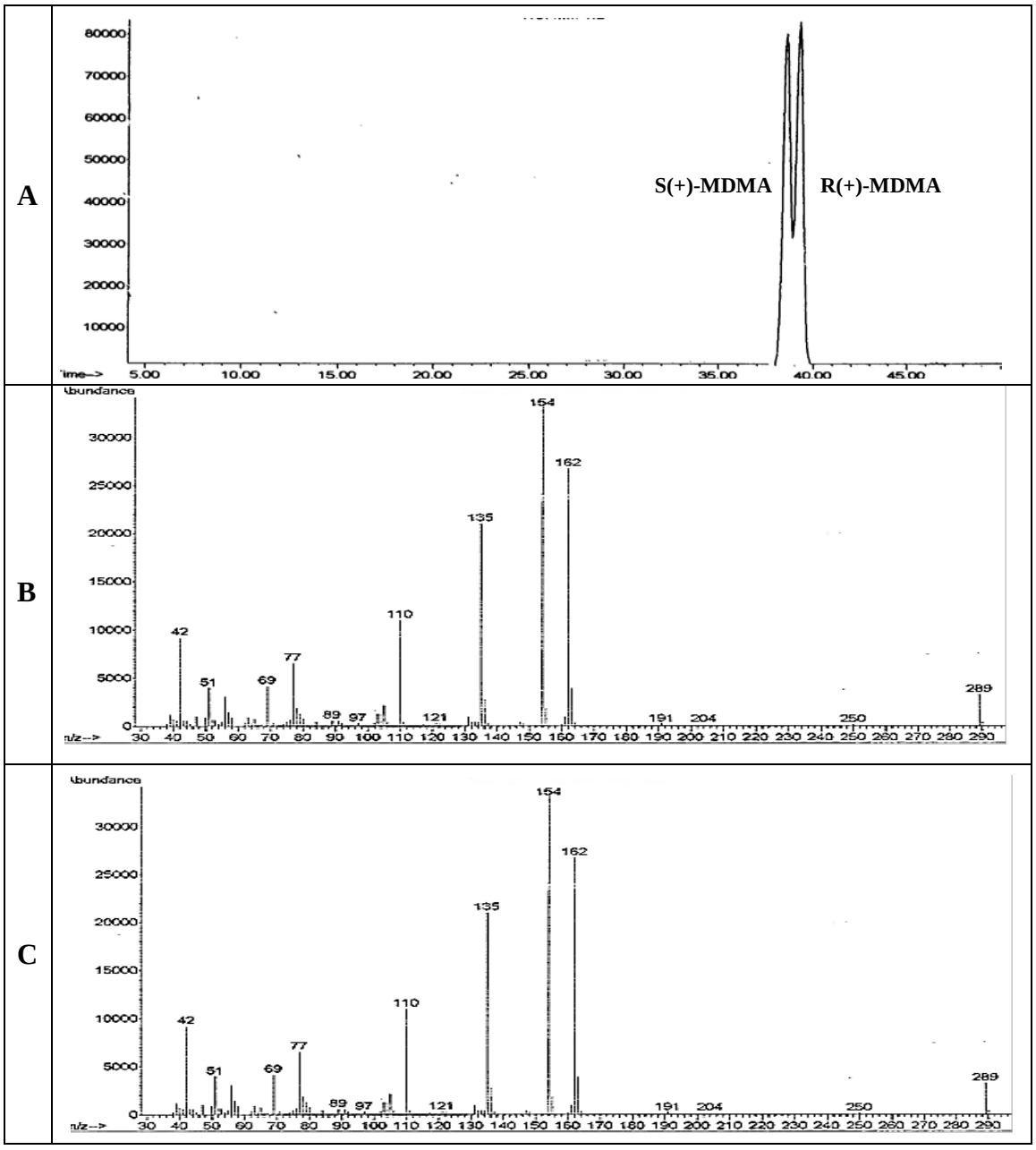


Figure 2.7 (A) The TIC of the TFAA derivatised MDMA control (1mg mL^{-1}). The peaks occurred at *Rts* of 38.5 and 39.4 min respectively. The EI mass spectra of (B) TFAA - S(+)-MDMA and (C) TFAA - R(-)-MDMA, both spectra having the same fragmentation pattern with base peak at m/z 154, ions at m/z 162, 135, 110 and 42 and molecular ion at m/z 289.

S(+)-MDMA enantiomer eluted slightly earlier then the R(-)-MDMA enantiomer. The mass spectrum of the two peaks, which were similar, had a base peak of m/z 154 with ions at m/z 162, 135, 110, 77 and 42 and a molecular ion at m/z 289 (Figures 2.5 and 2.6 respectively). The TFAA derivatised MDMA standard (Alltech, 1 mg mL^{-1}) had two

peaks with retention times of 38.5 (S(+)-MDMA) and 39.4 (R(-)-MDMA) min respectively. The EI mass spectra of the S(+)- and R(-)-MDMA from the tablet had both a base peak of m/z 154 with ions at m/z 162, 135, 110, 77 and 42 and a molecular ion at m/z 289 (Figure 2.7). The enantioseparation of the TFAA derivitised MDMA was partial.

To distinguish between an equal or non-equal ratio of enantiomers

A sample of 100 µl of racemic MDMA standard (1mg mL^{-1}, Alltech Standard) was derivatised and reconstituted as described above and analysed by GC-MS. The ratio of the enantiomers was determined as described above. This procedure was repeated for both single enantiomer samples S(+)- and R(-)-MDMA (concentration 1 µg μL^{-1}).

To 80 µL (80 µg) of the racemic MDMA standard were added 20 µL (i.e. 20 µg) of single enantiomer (S(+)- or R(-)-MDMA). The solution was derivatised by TFAA, using the method described above and then analysed by GC-MS. The difference in the isomeric content ratio when compared with the isomeric content ratio of the racemic MDMA sample was due to the added single enantiomer sample. The difference in the isomeric content ratios was due to the added single enantiomer control (Table 2.7, Figure 2.8).

Table 2.7 MDMA control and MDMA tablet after 'spiking' with the respective single enantiomer (peak area % of the enantiomers).

Racemic MDMA	Single enantiomer 20 µg	S(+)-MDMA peak area %	R(-)-MDMA peak area %	Ratio S(+)-/R(-)-
80 µg of MDMA control	S(+)-MDMA	59.69	40.31	~ 1.5 : 1
	R(-)-MDMA	40.63	59.37	~ 1 : 1.5
~72 µg of MDMA tablet – batch 19	S(+)-MDMA	60.78	39.22	1.55 : 1
	R(-)-MDMA	39.69	60.31	1 : 1.52

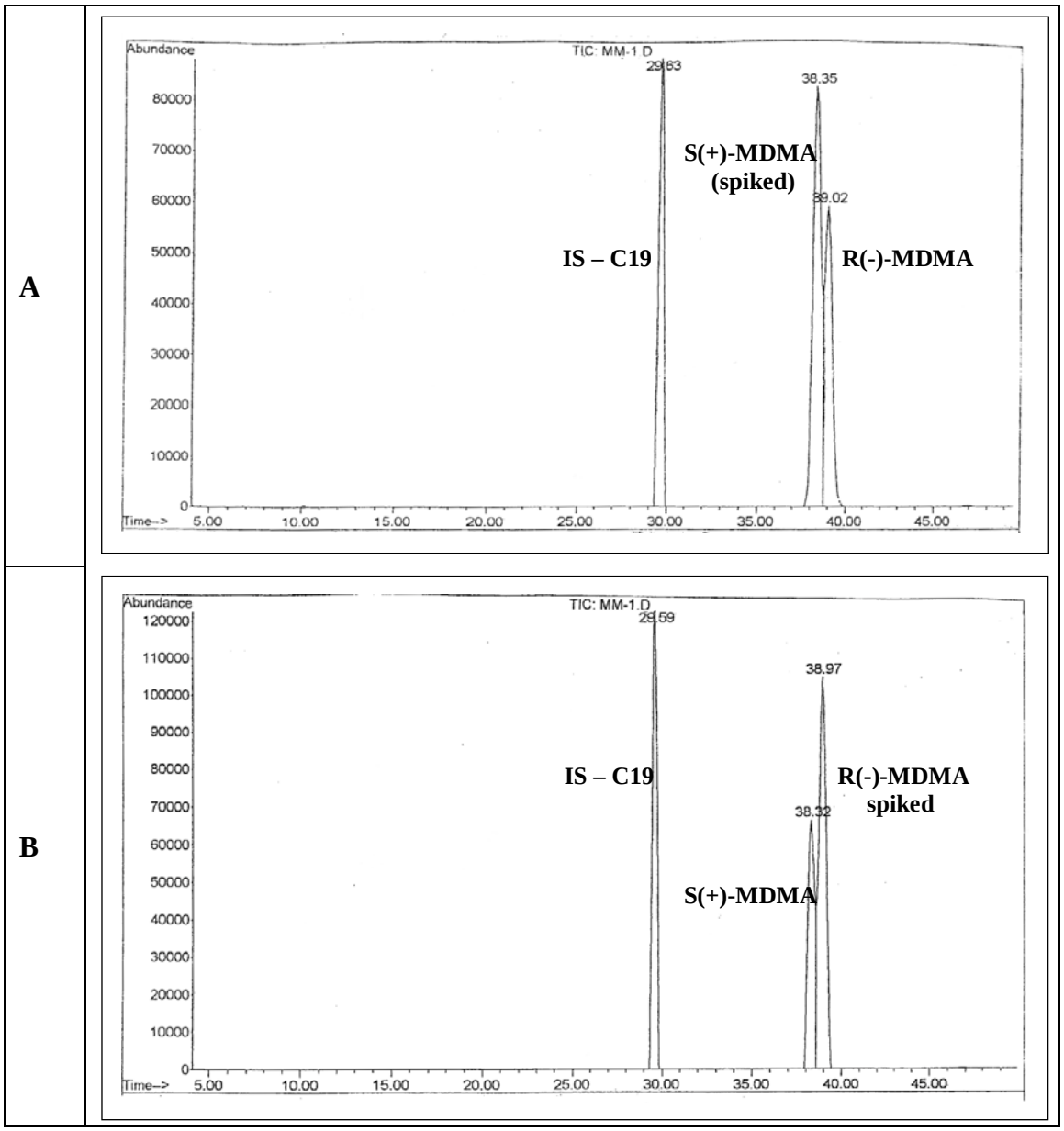


Figure 2.8 The TIC of (A) TFAA derivatised MDMA from tablet spiked with S(+)-MDMA control. Peaks at *Rt* 29.6, 38.4 and 39.0 min are attributed to internal standard (C19), TFAA - S(+)-MDMA from tablet (≈ 72µg) spiked with TFAA - S(+)-MDMA control (20 µg) and TFAA - R(-)-MDMA from tablet respectively; (B) TFAA derivatised MDMA from tablet spiked with R(-)-MDMA control (20 µg). Peaks at *Rt* 29.6, 38.3 and 39.0 min are attributed to internal standard (C19), S(+)-MDMA from tablet (≈ 72 µg) and R(-)-MDMA from tablet spiked with R(-)-MDMA control (20 µg) respectively.

To 80 µL (72 µg, from 0.9 mg mL^{-1}) of a racemic MDMA sample, from an 'ecstasy' tablet (batch 19, white coloured with heart logo), were added 20 µL of a 1 µg $µL^{-1}$ solution of the single enantiomer (S(+)- or R(-)-MDMA) and the resultant solution derivatised and analysed by GC-MS. The difference in the isomeric content ratios was due to the added single enantiomer control (Table 2.7, Figure 2.8).

The resolution of the enantiomers peaks was calculated using the equation:

$$R = 1.18 \left(\frac{t_{R2} - t_{R1}}{w_{h_1} + w_{h_2}} \right)$$

Where $\boldsymbol{t_{R2}}$ is the retention time of the first peak, $\boldsymbol{t_{R2}}$ is the retention peak of the second peak, $\boldsymbol{w_{h1}}$ is the peak width at half height (units of time) of the first peak and $\boldsymbol{w_{h2}}$ is the peak width at half height (units in time) of the second peak [235]. The resolution of the enantiomers peaks was ~ 0.79 (resolution 53%).

Robustness and stability

TFAA derivatised MDMA enantiomers controls were analysed with the GC oven at 150, 160 or 170°C and flow rates of 1, 2 and 5 mL min^{-1}. Robustness and stability of the GC analysis was checked by verifying if there was any change in the order of elution of enantiomers on the chiral stationary phase when the column temperature and carrier gas flow rate were varied at 5°C intervals between 150-165°C and flow rates of 0.5, 1 and 2 mL min^{-1}. No change was detected in the retention times of the derivatised single enantiomers. There was no separation of the enantiomers when the temperature of 165°C and flow rate of 0.5 mL min^{-1} were used.

Precision

Precision of analysis was determined as repeatability (intra-day) and intermediate

precision (inter-day). Repeatability was evaluated by assaying 6 determinations of TFAA derivatised MDMA standards at the same concentration (1µg µL^{-1}), during the same day, under the same experimental conditions. Intermediate precision was analysed by comparing the assay in 3 determinations at the same concentration (1µg µL^{-1}) on 3 separate days. Table 2.8 shows the intra-day and inter-day precision data of GC-MS method (TFAA derivatised MDMA standard, 1 µg µL^{-1}) for the percentage enantiomeric composition of S(+)- and R(-)-MDMA using software integration (Agilent Chemstation).

Table 2.8 Intra-day and inter-day precision data of GC-MS method for S(+)- and R(-)-MDMA enantiomers (RSD = relative standard deviation).

Precision	S(+)-MDMA %	R(-)-MDMA %	RSD
Intra-day - mean (*n* = 6)	**49.93**	**50.07**	**0.34**
Inter-day – day 1 - mean (*n* = 3)	49.93	50.07	0.22
Inter-day – day 2 - mean (*n* = 3)	50.02	49.98	0.14
Inter-day – day 3 - mean (*n* = 3)	49.99	50.01	0.24
Inter-day – mean (*n* = 9)	**49.98**	**50.02**	**0.20**
Intra and Inter-day – mean (*n* =15)	**49.96**	**50.04**	**0.26**

LoD and LOQ

LoD is the minimum concentration of (±) MDMA enantiomers which could still be detected by the analysis method and *LoQ* is the minimum concentration of (±) MDMA enantiomers that could still be quantitatively detected with accuracy and acceptable precision by the analysis method. These parameters were not a requirement for the (±) MDMA enantiomers determination percentage content; however, it is always useful to demonstrate that the analyses were being conducted in a region which was above the *LoQ* value. *LoD* and *LoQ* for each enantiomer were calculated from the calibration curve of 0, 25, 50, 100, 200 and 400 µL of (±) TFAA derivatised standard solution (1mg mL^{-1}), which contained 0 – 200 µg of both enantiomers. An internal standard C 19 was in each standard solution at a concentration of 25 µg mL^{-1}.

The *LoD* and *LoQ* were determined in the residual standard deviation and regression line ($y = 0.0078x + 0.0057$, $R^2 = 0.9981$ for both enantiomers) and slope. A plot of ratio peak area (Ax / As – peak are of analyte, Ax, is divided by the peak area of the internal standard, As) versus concentration (μg mL^{-1}) was drawn and *LoD* and *LoQ* values were predicted by STEYX (SD = 0.029) and slope (S = 0.0078) method using the formula 3.3 x SD / S for *LoD* and 10 x SD / S for *LoQ* [236]. The calculated values for *LoD* and *LoQ* were 12.27 and 37.18 μg mL^{-1} respectively.

2.2.3.3 Experiment C: Organic impurity profiling of MDMA-only tablets

The impurity profiling analyses were conducted to determine the possible synthetic routes of MDMA tablets. Moreover, the generated GC-MS impurity profiles were treated like fingerprints to link / discriminate the tablets taken from the different batches. The profiling method used was adapted from Gimeno et al. [121, 205]. A known mass of about 20 mg of crushed tablet was dissolved in 2 mL of water in a glass stoppered test-tube. The solution was made basic (pH 11.0) with the addition of 200 μL of 1 M NaOH and shaken for 10 min on an orbital shaker (Lab-Line, model 3520). Diethylether (3 mL) was added and the mixture shaken for another 10 minutes. The organic layer was then transferred to another test-tube by a Pasteur pipette.

The solvent was evaporated to dryness under nitrogen at room temperature and reconstituted in 500 μL of diethylether and then transferred to a micro-vial for profile analysis. In order to avoid the possibility of impurity degradation, 2 μL of the extracts were analysed by GC-MS on the same day they were prepared. Each sample was extracted and analysed in duplicate.

The GC-MS profiling analyses were carried on a Hewlett Packard 6890 GC coupled to a Hewlett Packard 5973 mass selective detector. The GC was fitted with an HP-5 (5% phenyl / 95% methylpolysiloxane) capillary column 30 m x 0.25 mm i.d., 0.25 μm film thickness and helium was used as a carrier gas at a flow rate of 1.0 min^{-1}. The injection (≈ 2μL) was made in splitless mode with GC injection port at 250°C. The GC oven

temperature was programmed as follows: 80°C for 2 min, 8°C min^{-1} to 210°C and 15°C min^{-1} to 300°C, with a 6 min final hold time. The mass spectrometer was operated in electron ionization mode (EI) electron energy 70 eV. The temperature of the MS source was 230°C, MS quadrupole 150°C and transfer line 280°C. A full scan mass spectrum 40-500 amu was obtained at a scan rate of 0.3 scan s^{-1}.

The EI mass spectra of the compounds were identified by either using spectra published in the literature or by the use of the National Institute of Standards and Technology library PMW_TOXR.L.

2.2.3.4 Experiment D: Determination of major excipients in 'ecstasy' tablets by Fourier transform infrared (FTIR) transmittance spectroscopy

Major excipients in these set of experiments refers to diluents which are added to increase the bulk volume when the amount of the active ingredient is insufficient to form a tablet of acceptable size. In order to investigate the major excipients in 'ecstasy' tablets a single tablet from each of the 34 batches (34 not 45 batches were used as tablets from the other batches were all used) was used and an FTIR spectrum of the powdered tablet was obtained using an Interspec 2020 FTIR spectrometer. The tablet sample was prepared by grinding and thoroughly mixing about 1 mg of the sample with about 300 mg of spectral grade potassium bromide (KBr). The background was spectral grade KBr. Both the crushed tablets and the KBr were kept in an oven at 40^{o}C overnight before preparing the KBr. KBr disk spectra were recorded between 4000 and 500 cm^{-1}, by averaging 32 scans for each spectrum at a resolution of 4 cm^{-1}. The spectrum of the excipient lactose is produced.

2.2.3.5 Experiment E: Elemental profiling of 'ecstasy' tablets by scanning electron microscopy with energy dispersive X-ray analyser (SEM/EDX)

Analyses were conducted on 37 tablets taken from batches (37 not 45 batches were used

as tablets from the other batches were all used). The tablets were each mounted on a SEM sample stub using double-sided carbon adhesive tape. The tablets, which were coated with a thin carbon layer in a sputter coater (Emiscope, UK), were subjected to elemental analysis using a SEM (Zeiss Evo 50, Oberkochen Germany) and EDX detector (Oxford Inca 200, Oxford Instruments, Wiesbaden Germany). The EDX was equipped with a liquid nitrogen cooled x-ray detector (Si(Li) having 10 mm^2 crystal. A fixed working distance of 16 $\pm$ 1 mm for the EDX detector and electron energy (acceleration voltage) of 25 kV were used. A fixed magnification of 25 times and a live time of 50 seconds were used during EDX analyses. The spatially resolved semi-quantitative (sample was not polished) EDX analysis of the tablet area ($\approx$ 7 mm^2) was obtained by the detection of characteristic X-rays that were emitted by the tablet following excitation by a high energy electron beam. The X-ray spectra were optimized by a cobalt optimization standard.

SEM/EDX method precision

The precision was determined by repeatability (intra-day) and intermediate precision (inter-day). Repeatability was evaluated by assaying an 'ecstasy' tablet at 8 different positions in the SEM chamber, using the rotating specimen stage, during the same day, under the same experimental conditions. An area of $\approx$ 7 mm^2 of the tablet was analysed each time with the exception of the intra-day analyses of 10 random spots on the surface of the tablet. Intermediate precision was analysed by comparing the same 'ecstasy' tablet during 10 different days at the same position on the rotating specimen stage in the SEM chamber, using the same experimental conditions. Precision (repeatability and intermediate precision) was expressed as relative standard deviation (RSD) of the mean (SD/mean*100%).

The precision for the SEM/EDX method was conducted on a white tablet with euro logo (batch 11). Table 2.9 shows the mean weight % for the elemental ions, Mg, Si and Cl detected by SEM/EDX analyses for the tablet from batch 11. The mean weight % for the individual elemental ions was quite high with the quantification method used.

Table 2.9 Intra-day and inter-day precision data of the weight % of elemental ions Mg, Si and Cl detected on a tablet, containing MDMA, from batch 11 (white tablet with euro logo) by SEM/EDX analyses.

Elemental ions – W eight %			
Precision (Tablet no. 2 MDMA with euro logo)	**Mg**	**Si**	**Cl**
Intra-day (*n* = 8 positions) **(analysed area ≈ 7 mm^2)**	**17.57 (14.00)**	**22.31 (16.74)**	**60.12 (9.78)**
Intra-day (*n* = 8 positions) **(analysed 10 random spots)**	**18.46 (17.93)**	**21.74 (19.55)**	**59.80 (10.99)**
Inter-day - (*n* = 3 positions) (analysed area ≈ 7 mm^2) - day 1	18.21	24.19	57.60
Inter-day - (*n* = 3 positions) (analysed area ≈ 7 mm^2) - day 2	15.23	19.45	65.32
Inter-day - (*n* = 3 positions) (analysed area ≈ 7 mm^2) – day 3	17.45	21.44	61.11
Inter-day - (*n* = 3 positions) (analysed area ≈ 7 mm^2) – day 4	20.56	20.73	58.71
Inter-day - (*n* = 3 positions) (analysed area ≈ 7 mm^2) – day 5	18.78	26.63	54.59
Inter-day (*n* = 15) [a]	**18.05 (10.80)** [b]	**22.49 (12.85)**	**59.46 (6.78)**

[a] ***n = 15 is the total number of positions after 5 days (3 positions analysed each day).***
[b] ***The numbers are the means of the weight % and the numbers in parentheses are the RSDs.***

The variation, represented by the RSD, for the detected elemental ions was higher for the method analyzing 10 spots at eight different positions of the tablet, than for the method analysing an area of ≈ 7 mm^2 at eight different positions (Table 2.9). Hence, the second method, where an area of ≈ 7 mm^2 of the tablet was analysed, was used for the elemental profiling of the tablets. However, the RSD for the second method was still high and therefore it was considered as semi-quantitative.

Variation within batch

The weight % variation (represented by the RSD) of the detected individual elemental ions for a batch of tablets was determined by analysing six tablets, taken at random, from

Table 2.10 SEM/EDX analyses of an area of ≈ 7 mm^2 of 6 white tablets, containing MDMA, from batch 29 (D&G logo). The data shows the weight % of elemental ions Mg, Al, Si and Cl detected on the tablets (numbers in brackets are the relative standard deviation, RSD).

Batch 29 Elemental ions Weight %	Mg	Al	Si	Cl
Tablet 1	21.01	6.95	21.06	50.98
Tablet 2	19.65	8.67	23.41	48.27
Tablet 3	22.85	5.97	17.79	53.39
Tablet 4	20.06	8.30	20.53	51.11
Tablet 5	17.34	7.94	23.91	50.81
Tablet 6	21.23	8.54	23.60	46.63
Mean	**20.36**	**7.73**	**21.72**	**50.19**
RSD	**(9.09)**	**(13.71)**	**(11.00)**	**(4.76)**

Table 2.11 SEM/EDX analyses of an area of ≈ 7 mm^2 of 6 white tablets, containing mCPP, from batch 32 (mercedes logo). The data shows the weight % of elemental ions Mg, P, Cl and Ca detected on the tablets (numbers in brackets are the relative standard deviation, RSD).

Batch 32 Elemental ions Weight %	Mg	P	Cl	Ca
Tablet 01	2.89	34.64	21.24	41.23
Tablet 02	2.61	38.76	19.24	39.39
Tablet 03	3.34	37.63	20.58	38.45
Tablet 04	3.56	36.51	21.92	38.01
Tablet 05	3.82	44.43	16.66	35.09
Tablet 06	3.69	38.93	21.01	36.37
Mean	**3.31**	**38.48**	**20.11**	**38.09**
RSD	**(14.50)**	**(8.63)**	**(9.50)**	**(5.72)**

two batches of 'ecstasy' tablets, batch 29 (containing MDMA tablets), Table 2.10 and batch 32 (containing mCPP tablets), Table 2.11. These two batches (batches 29 and 32), containing different psychoactive substances, were also chosen to compare any difference in their intra-batch variation. However, not much difference was noted when the results for the intra-batch variation for detected elemental ions for the respective batches were compared.

SEM/EDX – weight percentage calculation of detected elements

The Oxford INCA software in the EDX was used to calculate the weight percentage of the detected elements (software suppresses the background). Data of the default standard and detected peak intensity of selected sample element were used to calculate the apparent concentration using the equation:

$$\textbf{Apparent concentration of A} = (IA_{spl} \div IA_{std}) \times wt\%A_{std}$$

Where IA_{spl}, IA_{std} and $wt\%A_{std}$ are the detected intensity of element A in the tablet and the default intensity of element A in the standard respectively. The apparent concentration was then subjected to intensity correction to give the weight percentage of each element. Finally, the total percentage of these selected elements was adjusted to 100% to give a normalized result.

2.2.4 Analyses of substances seized during EDM parties

Qualitative analyses were conducted on the powders, plants and cannabis-like material. Pharmaceutical tablets, which were identified visually and compared to an in-house tablets database, were not analysed unless their identity was in question.

2.2.4.1 Colour tests

The powders and liquids seized during EDM parties were first screened by means of colour tests as described in Section 2.2.3.1 to determine the presence or absence of active ingredients. The plant like material was screened by the Duequenois-Levine reagent for the presence of THC. The Duequenois-Leivine reagent was prepared as follows:

- ***Duequenois-Leivine reagent [232]:***

  Solution 1 was prepared by dissolving 0.4g of vanillin in 20 mL of 95% ethanol and then adding 5 drops of acetaldehyde. Solution 2 was concentrated hydrochloric acid and solution 3 chloroform.

2.2.4.2 Thin layer chromatography (TLC)

TLC was also used to determine the presence or absence of heroin, cocaine and THC. The following TLC systems were used:

Heroin and cocaine

Powders (≈ 5 mg mL^{-1}) from samples were dissolved in chloroform. Test solutions, positive and negative controls (negative control: chloroform; positive controls: heroin and cocaine at 1mg mL^{-1}) were developed on a silica gel 60 F_{254}, 0.25 mm pre coated TLC plate (Whatman Ltd. Maidstone, UK) using a mobile phase of chloroform : methanol solution (90:10 v/v). Detection was achieved by spraying with acidified iodoplatinate solution [232].

Cannabis

The material (herbal - ≈ 1g or resin - ≈ 0.25 g) was pulverized and extracted using chloroform to produce a sample concentration of 50 mg mL^{-1} for the herbal and ≈ 5 mg

mL^{-1} for the resinous material. Test solutions, positive and negative controls (negative control: chloroform; positive control: THC at 100 μg mL^{-1}) were developed on a silica gel 60 F_{254}, 0.25 mm precoated TLC plate (Whatman Ltd. Maidstone, UK) using a mobile phase of petroleum spirit 60/90 : diethyl ether (80:20 v/v) for separation. Detection was achieved by fast blue B spray (50 mg of fast blue B dissolved in 1 mL of water and 20 mL of methanol) [237].

2.2.4.3 Confirmation of active ingredients by GC-MS

Methanoic extracts were prepared for the powders, plants and cannabis-like material with a final concentration of ≈ 1 mg mL^{-1}. The powders were assumed to be ≈ 50% pure (usually cocaine), while the plants and cannabis-like material was assumed to be ≈ 10% pure. For the liquid, suspected to be gamma-butyrolactone (GBL), a 0.1% solution in chloroform was prepared. The same GC-MS method described in Section 2.2.3.1 was used to determine the active ingredients in the seized material.

2.3 Court Permission

In Malta the appointment of a forensic drug analyst by the magistrate falls under the provisions of article 650 (1) of Chapter 9 of the Criminal Code, which states that "in all cases where for the examination of any person or thing special knowledge or skill is required, a reference to experts shall be ordered**"** [238]. All drug analyses on the Island are carried out at the Forensic Laboratory Services which makes part of the Malta National Laboratory (MNL). The appointed court forensic analyst would do the analysis and would then report his findings to the Courts in *viva voce* in front of the accused.

All the 'ecstasy' tablets that were physically and chemically profiled in this research study were obtained from court cases where tablets were seized from traffickers or users by the Police Drug Squad. In all cases the researcher has obtained the necessary permissions from the inquiring magistrates to use a sample of the seized tablets for the research study. In the study to monitor illegal substances for personal use (Chapter 6A),

samples were confiscated by the police from partygoers at major EDM parties and those where the researcher was the appointed court expert were included in the analyses.

2.4 Statistical Methods

All calculations were performed on the Statistical Package for Social Science (SPSS) version 16.

2.4.1 Physical features of tablets and stability studies

Descriptive statistics were used in these studies. The examined tablets in the physical characterisation study were selected at random from the batches using the hypergeometric method. One-way analyses of variance (ANOVA), using a p value of 0.05 as a cut-off for statistical significance, was used in the physical characterisation and stability studies. In the physical characterisation study one-way ANOVA was used to compare the means for each measurable feature (mass, diameter, thickness, hardness and disintegration rate) in order to discriminate or link batches of 'ecstasy' tablets. In the stability studies one-way ANOVA was used to test the difference in the physical features of tablets after a photostability experiment and two other experiments were tablets were subjected to various temperatures and humidity

2.4.2 Drugs seized at EDM parties and the Malta party study

Descriptive statistics were used in these studies. For the drugs seized at EDM parties and the Malta party study the data was statistically tested using the one-way ANOVA. When the normality and homogeneity of variance assumption was not satisfied the equivalent non-parametric Kruskall-Wallis test was applied. Post hoc tests for ANOVA were Tukey HSD and Games-Howell. The Games-Howell procedure was used when the variances were unequal when tested with Levene's test for equality of variances. Significance was set at $p < 0.05$ for all tests.

Chapter 3

PHYSICAL CHARACTERISTICS OF 'ECSTASY' TABLETS

'Ecstasy' tablets normally provide visual and physical characteristics that are usually produced during the tabletting process [144]. While the increase in the misuse of 'ecstasy' had prompted scientists to use these features to compare illegal tablets, in the literature the physical features of 'ecstasy tablets have been neglected [181].

3.1 Introduction

The presence of physical features of 'ecstasy' tablets could provide valuable information that potentially can link tablets from different seizures [239]. Some researchers claim that tablets with similar physical features are from the same production batch, while tablets with different characteristics were from different batches but that these physical features were not considered sufficient to provide evidence of a link between seizures in court [214]. However, others argue that tablets with the same physical characteristics do not necessarily mean that these have the same chemical compositions [240]. A further view is that some clandestine laboratories use different metal taps and dies to produce 'ecstasy' tablets having the same active ingredient but with different visual characteristics [212].

One of the determinants by which illegal drugs are used is their perceived quality and price [241, 242]. It is claimed that illicit drug users are willing to pay more money to acquire better quality controlled drugs and they would only pay for drugs when they think that the quality is acceptable [242]. Hence, the visual and the physical features of 'ecstasy' tablets could be of importance to users if tablets give the perception of 'good' quality drugs, thus making the user decide to buy the tablets. However, while some hypothesised that the decrease in the perceived quality of 'ecstasy' would have a

significant impact on the number of users who purchase these tablets and the amount purchased, others claimed the opposite stating that the decrease in quality would have no such effect [242, 243].

The aim of this part of the research study was to investigate the distinguishing features (visual and measurable features) of seizures collected by the police authorities over a five-year period, from 2006 - 2011, to determine if these features could be used to discriminate or link batches for drug intelligence purposes. Within this study discrimination and linking is defined as the ability to differentiate or connect batches of 'ecstasy' tablets by their visual (logo, shape, breakline and colour) and / or measurable (mass, diameter, thickness, hardness, friability and disintegration rate) features. In addition, the data obtained from the measurable features was also compared against standards set for pharmaceutically produced tablets to determine how "well-made" the 'ecstasy' tablets seized in Malta during a five year period. The study also sought to test the following ***hypothesis (1)***, that:

> ***The physical state of different batches of 'ecstasy' tablets seized on different occasions in Malta during 2006 – 2011 will be significantly different from each other.***

3.2 Methods

In this study, a total of 30 seizures that comprised of 45 batches of 'ecstasy' tablets were examined for their visual and measurable physical characteristics. Table 3.1 records the number of tablets in each batch and the date of the seizure. The visual features of the ecstasy' tablets, which included the logo, tablet shape, breakline (if any) and colour, described in the methodology in Section 2.2.1.1, were identified.

All the measurable physical characteristics of the samples of tablets, which were taken at random from each batch, were evaluated together against the pharmacopoeial or

pharmaceutical criteria listed below (mass, diameter, thickness, hardness, friability and disintegration rate).

3.2.1 Mass, diameter and thickness

The measurements for the mass, diameter and thickness were made on randomised samples of tablets from each batch as described in the methodology Section 2.2.1.3. The following criteria were applied:

a) ***Uniformity of mass***: that not more than 2 of the individual masses of tablets should deviate from the average mass by more than 7.5% for tablets with average mass of more than 80 mg and less than 250 mg, and by not more than 5% for tablets with average mass of more than 250 mg [221];

b) ***Diameter***: British Pharmacopoeia criteria that the diameter of tablets with diameter up to 12.5 mm can deviate by up to 5% [225];

c) ***Thickness***: the 5% tolerance as for normal manufacturing practices [244];

3.2.2 Hardness (mechanical strength), friability and disintegration

The physical tests with limits of acceptance, relating to the tablet performance of handling, hardness and friability, and disintegration were carried according to the methods as described in the methodology Section 2.2.1.3. The applied criteria were:

a) ***Hardness***: applied for pharmaceutical tablets - 39.22 to 78.45 N [245];

b) ***Friability***: loss in mass of less than 1% [221];

c) ***Disintegration rate***: rate at which tablets would disintegrate in 900 s (15 min) [225].

Table 3.1 The 45 batches of 'ecstasy' tablets from 30 cases, seized by the Malta Police Drug Squad from 2006 - 2011, that were used for physical characterisation.

Batch No.	Tablet	Year Seizure No.	No. of Tablets in Batch	Batch No.	Tablet	Year Seizure No.	No. of Tablets in Batch	Batch No.	Tablet	Year Seizure No.	No. of Tablets in Batch
1		2006 1	400	**16**		2007 4	1,379	**31**		2008 19	1,000
2		2006 2	69	**17**		2007 5	1,837	**32**		2008 20	538
3			100	**18**		2007 6	2,195	**33**		2009 21	837
4		2006 3	50,579	**19**		2007 7	2,400	**34**		2010 22	6,926
5			1,021	**20**		2007 8	1,990	**35**			1,947
6			1,018	**21**		2007 9	2,876	**36**		2010 23	590
`7			1,154	**22**		2007 10	4,989	**37**			1,261
8			1,029	**23**		2008 11	172	**38**		2010 24	365
9			1,142	**24**		2008 12	153	**39**			1,124
10		2007 4	1,032	**25**		2008 13	124	**40**		2010 25	1,000
11			1,069	**26**		2008 14	154	**41**		2011 26	369
12			1,020	**27**		2008 15	103	**42**		2011 27	281
13			1,031	**28**		2008 16	124	**43**		2011 28	194
14			1.114	**29**		2008 17	133	**44**		2011 29	113
15			1,344	**30**		2008 18	108	**45**		2011 30	236

Data analysis

In this study one-way ANOVA was used in order to discriminate or link batches of 'ecstasy' tablets as described in Section 2.4.1.

3.3 Results

The visual and measurable physical characteristics of the 30 seizures made up of 45 batches of 'ecstasy' tablets (98,640 tablets) are shown in Table 3.2, which displays the logo and tablet shape, breakline (if any) and colour. While most of the seizures consisted of 1 batch of tablets, seizures 2, 22, 23 and 24 consisted of 2 batches each and seizure 4 consisted of 12 batches.

3.3.1 Visual characteristics of MDMA / ecstasy tablets

All the examined batches of 'ecstasy' tablets had tablets (about 60 to 70%) with defects, such as mottling, chipping, sticking and capping among others. Some of the batches of tablets had more defects than others such as batches 11-13, the white euro logo tablets, which were chipped, and tended to cap, while batches 27, the blue with question mark logo tablets, 42, the blue with euro logo tablets and 43, the blue with armani logo tablets were very mottled.

The visual characterisation of the 45 batches of 'ecstasy' tablets are given in Table 3.2., including those for the control bumetanide tablets.

3.3.1.1 Logos and shape

From the 45 batches of tablets from the 30 seizures it was found that most of the batches had round shaped tablets with logos (91%, n = 41 respectively). Four of the batches had non-round-shaped tablets; two batches with heart shaped tablets, one batch with diamond shaped tablets and another batch with triangular shaped tablets. The most common logos were the omega and euro 6 batches each (batches 5-10 and 11-13, 17, 24 and 42), heart 4 batches (batches 19, 28, 36 and 37), followed by the lacoste, pisces and question mark 3 batches each (batches 4, 44 and 45 for lacoste, 14-16 for pisces and 27, 34 and 35 for question mark) (Table 3.2), which together these made up 56% of the tablets examined.

Table 3.2 Visual characteristics of 45 batches of 'ecstasy' tablets from 30 cases.

Batch No.	Tablets	Logo	Colour	Breakline	Batch No.	Tablets	Logo	Colour	Breakline
0		bumetanide control	white	yes	**23**		/	pink	no
1		thumbs-up	blue	no	**24**		Euro	blue	no
2		/	white	no	**25**		Star	white	yes
3		/	white	no	**26**		Smiley	pink	no
4		lacoste	white	yes	**27**		question mark	blue	yes
5		omega	blue	yes	**28**		Heart	white	yes
6		omega	blue	yes	**29**		D&G	white	no
7		omega	blue	yes	**30**		/	blue	yes
8		omega	green	yes	**31**		Tulip	white	no
9		omega	green	yes	**32**		Mercedes	white	no
10		omega	green	yes	**33**		Versace	white	yes
11		euro	white	no	**34**		question mark	blue	no
12		euro	white	no	**35**		question mark	green	no
13		euro	white	no	**36**		Heart	blue	no
14		pisces	orange	yes	**37**		Heart	blue	no
15		pisces	orange	yes	**38**		rolex crown	blue	no
16		pisces	orange	yes	**39**		rolex crown	green	no
17		euro	white	yes	**40**		$E=mc^2$	white	no
18		D&G	white	no	**41**		route 66	white	cross
19		heart	white	no	**42**		Euro	blue	no
20		shark	white	yes	**43**		Armani	blue	no
21		kangaroo	pink	no	**44**		Lacoste	pink	cross
22		letter X	white	no	**45**		Lacoste	pink	cross

3.3.1.2 Breaklines

More than half (55.6%, n = 25) of the batches of 'ecstasy' tablets did not have a

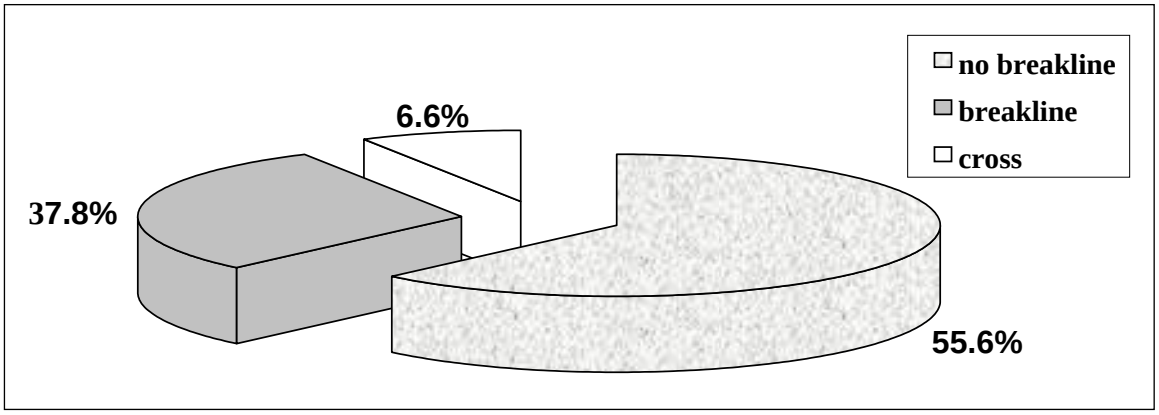


Figure 3.1 Breaklines on 'ecstasy' tablets for all years (2006 - 2011).

breakline. A breakline aligned along the diameter of the tablet was found in 17 batches (37.7%), while the other remaining batches had a cross breakline (Figure 3.1).

3.3.1.3 Tablet colour

In an attempt to minimize the problems that occur when trying to classify tablets by colour, shades of colour were not recorded. Thus tablets which appeared light blue were classified only as 'blue'. Figure 3.2 shows the percentage frequency of colours within the examined 45 batches.

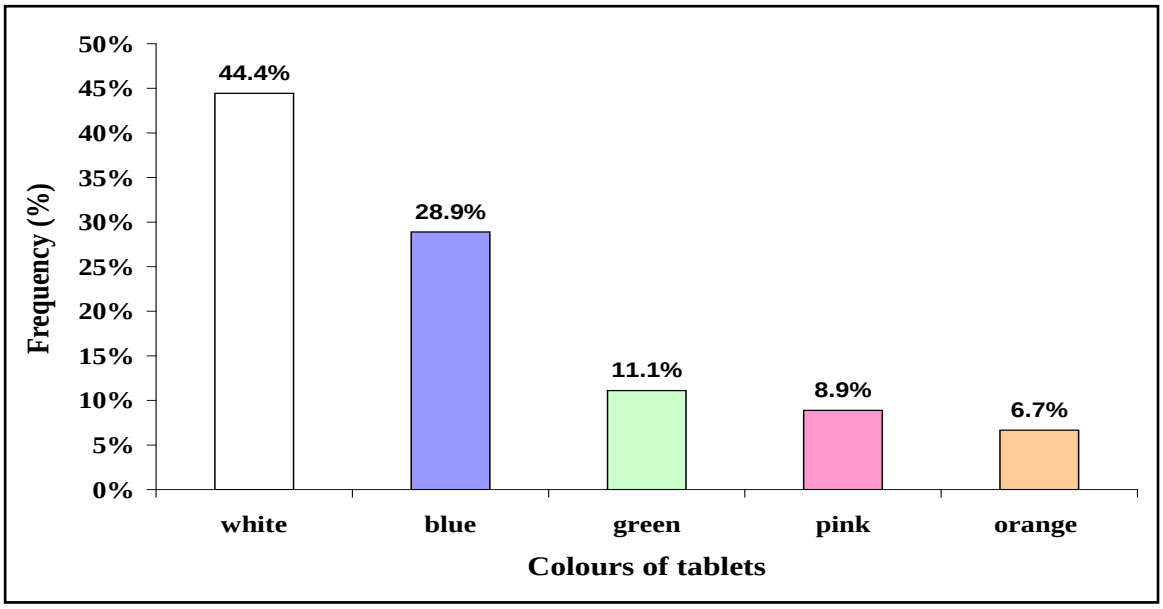


Figure 3.2 Colours of the examined 'ecstasy' tablets in percentage frequency.

Most (44%, n = 20 batches) of the tablets were white followed by blue (29%, n = 13) which together these made up 73% of tablets examined. During the period 2009 - 2011 more batches of coloured tablets (76.9%, n = 10 out of 13 batches) were seized.

The physical characteristics of the tablets including, mass, diameter and thickness of the 45 batches of 'ecstasy' tablets are given in Table 3.3. The bumetanide tablets were control tablets.

3.3.2 Physical characteristics: mass, diameter and thickness

3.3.2.1 Mass

The means of the mass of tablets in each of the 45 batches ranged between 135 mg to 448 mg and the RSD values of the majority (93.3%, n = 42) of the batches were under 5% . While the mean of means of the mass of the batches was 237.2 mg, the median was 236 mg (RSD 28.9% and RQD 22.7%, Table 3.3, Figure 3.3). However, over the five year period the mean mass of most batches ranged between 200 mg to 300 mg (71%, n = 32). The bumetanide control tablets (n = 29) had a mean mass of 177 mg and the RSD was 0.91%.

From the uniformity of mass test it transpired that most (73%, n = 33) of the batches of 'ecstasy' tablets had no more than 2 tablets that deviated from the mean mass by more than 5% (tablets with mean mass > 250 mg) or 7.5% (tablets with mean mass > than 80mg but < 250 mg). The rest of the batches of tablets had more than 2 tablets that deviated from the mean by more than 5% or 7.5%.

Difference was also noted in the intra-batch variation in tablet mass for the respective batches, such as the 27 blue tablets with the rolex crown logo (batch 38, mean mass of 338 mg, RSD 1.31%), with low intra-batch variability, the 29 pink tablets with the kangaroo logo (batch 21, mean mass of 241 mg, RSD 3.39%), with median intra-batch variability and the 28 white tablets with $E = mc^2$ logo (batch 40, mean mass of 237 mg,

Table 3.3 Physical characteristics, mass, diameter and thickness of 45 batches of 'ecstasy' tablets from 30 cases.

Batch No.	Tablets	Mass mg	Diameter mm	Thickness mm	Batch No.	Tablets	Mass Mg	Diameter mm	Thickness mm
0		177 (0.91) 176 (0.57)	8.12 (0.25) 8.12 (0.19)	2.73 (1.10) 2.73 (1.28)	23		135 (3.85) 137 (5.13)	7.08 (0.10) 7.08 (0.14)	3.31 (3.04) 3.31 (2.72)
1		279 (3.91) 281 (6.58)	7.65 (0.81) 7.77 (0.72)	3.86 (4.14) 3.89 (4.15)	24		202 (1.46) 203 (2.47)	7.13 (0.14) 7.13 (0.07)	3.59 (0.68) 3.59 (0)
2		432 (4.19) 429 (6.99)	11.30 (0.17) 11.30 (0.09)	6.10 (2.95) 6.10 (3.44)	25		194 (2.71) 194 (3.35)	8.10 (0.33) 8.09 (0.43)	3.45 (3.62) 3.49 (5.44)
3		448 (3.39) 449 (5.79)	9.20 (0.33) 9.21 (0.54)	6.30 (3.13) 6.38 (3.92)	26		209 (3.00) 211 (2.61)	8.20 (0.81) 8.21 (0.31)	3.28 (1.80) 3.29 (1.82)
4		280 (4.93) 279 (5.38)	9.10 (0.69) 9.08 (0.11)	3.50 (4.97) 3.48 (6.32)	27		258 (4.46) 258 (5.81)	7.11 (0.18) 7.12 (0.21)	5.34 (2.57) 5.34 (3.09)
5		240 (4.65) 245 (7.16)	9.22 (0.55) 9.20 (0.78)	3.14 (6.66) 3.15 (6.61)	28		236 (2.79) 236 (4.03)	8.40 (0.29) 8.40 (0.36)	5.08 (2.56) 5.08 (1.97)
6		239 (3.32) 241 (6.33)	9.21 (0.47) 9.20 (0.47)	3.09 (3.46) 3.11 (5.76)	29		197 (1.71) 198 (2.78)	7.05 (0.09) 7.05 (0.14)	3.97 (0.88) 3.97 (1.01)
7		240 (3.66) 243 (2.98)	9.21 (0.56) 9.21 (0.68)	3.12 (3.65) 3.14 (3.08)	30		135 (4.00) 135 (6.30)	7.06 (0.31) 7.05 (0)	3.27 (2.60) 3.24 (3.55)
8		239 (4.02) 240 (4.70)	9.20 (0.53) 9.19 (0.47)	3.07 (4.76) 3.09 (3.91)	31		206 (3.14) 206 (5.11)	7.10 (0.18) 7.10 (0.18)	3.20 (1.47) 3.20 (1.96)
9		236 (4.21) 237 (4.65)	9.22 (0.45) 9.23 (0.50)	3.09 (3.53) 3.10 (3.27)	32		222 (1.45) 223 (1.79)	7.08 (0.09) 7.08 (0.14)	3.27 (1.47) 3.28 (0.76)
10		236 (4.34) 239 (4.40)	9.21 (0.52) 9.20 (0.65)	3.05 (4.49) 3.10 (4.75)	33		274 (2.23) 275 (1.46)	8.19 (0.30) 8.19 (0.24)	4.39 (1.50) 4.39 (1.71)
11		201 (3.22) 200 (3.63)	7.13 (0.10) 7.12 (0.13)	4.11 (3.31) 4.15 (2.13)	34		179 (2.24) 179 (2.79)	7.09 (0.17) 7.08 (0.14)	3.61 (2.69) 3.59 (3.34)
12		202 (4.59) 204 (5.39)	7.13 (0.10) 7.13 (0.08)	4.15 (3.25) 4.17 (3.15)	35		177 (3.25) 175 (5.14)	7.09 (0.71) 7.08 (0.42)	3.29 (3.68) 3.29 (6.38)
13		200 (4.19) 203 (5.17)	7.13 (0.17) 7.13 (0.18)	4.15 (3.25) 4.17 (2.59)	36		201 (3.00) 201 (5.11)	7.12 (0.14) 7.12 (0.14)	3.95 (3.54) 3.90 (5.78)
14		154 (3.38) 154 (4.55)	7.07 (0.18) 7.07 (0.19)	3.86 (3.36) 3.85 (5.03)	37		333 (1.82) 333 (3.15)	9.21 (0.15) 9.20 (0.22)	4.50 (1.51) 4.50 (1.78)
15		154 (3.47) 155 (5.66)	7.12 (0.21) 7.12 (0.23)	3.95 (3.80) 3.99 (5.47)	38		338 (1.31) 338 (1.78)	9.19 (0.09) 9.20 (0.11)	4.34 (2.42) 4.34 (4.38)
16		154 (2.49) 154 (2.60)	7.11 (0.18) 7.11 (0.29)	3.87 (3.88) 3.83 (5.46)	39		364 (1.44) 365 (1.99)	9.32 (0.24) 9.31 (0.22)	4.19 (1.50) 4.20 (1.73)
17		211 (2.22) 211 (2.84)	8.09 (0.21) 8.09 (0.31)	4.37 (1.37) 4.39 (0.48)	40		237 (10.89) 234 (18.42)	9.09 (0.36) 9.09 (0.50)	3.04 (9.44) 3.01 (15.03)
18		200 (3.57) 198 (5.56)	7.02 (0.09) 7.02 (0.11)	4.13 (1.21) 4.13 (1.19)	41		246 (6.58) 245 (6.94)	9.08 (0.17) 9.08 (0.06)	3.36 (5.48) 3.34 (7.19)
19		202 (3.22) 202 (4.46)	7.10 (0.99) 7.10 (0.14)	4.38 (1.37) 4.39 (0.16)	42		198 (1.95) 199 (2.02)	7.09 (0.49) 7.08 (0.39)	3.99 (1.00) 4.00 (0.94)
20		295 (4.46) 296 (6.42)	9.10 (5.17) 9.16 (4.15)	3.95 (2.63) 3.95 (0.96)	43		203 (3.88) 203 (3.94)	7.11 (0.31) 7.11 (0.28)	3.96 (1.94) 3.97 (1.76)
21		241 (3.39) 244 (2.46)	8.09 (0.25) 8.09 (0.10)	3.58 (1.96) 3.60 (2.11)	44		249 (8.42) 241 (8.51)	9.20 (0.12) 9.20 (0.16)	3.48 (6.61) 3.39 (7.52)
22		352 (3.64) 352 (5.68)	/	4.43 (3.61) 4.32 (3.16)	45		248 (8.27) 244 (7.79)	9.20 (0.15) 9.20 (0.19)	3.48 (7.04) 3.39 (7.76)

Note: *Mass, diameter and thickness – 1st line: mean, and relative standard deviation (RSD), 2nd line: median and relative quartile deviation (RQD), for batches 2, 23 and 30 for the diameter the length was measured).*

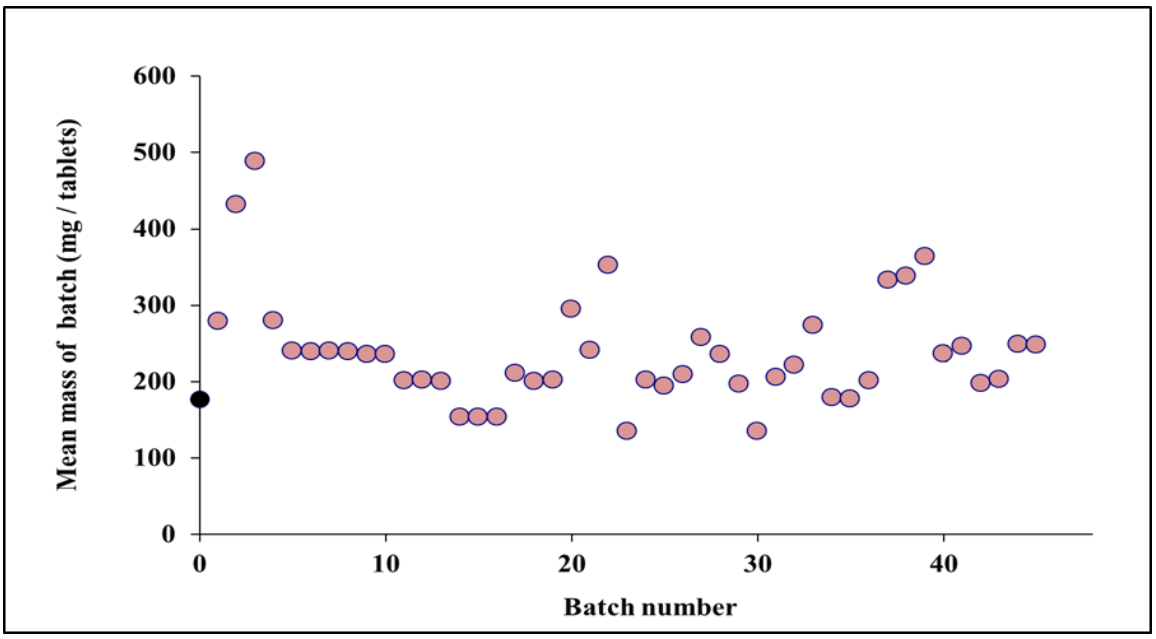


Figure 3.3 Mean mass (mg) of each batch of 'ecstasy' tablets over a 5 year period from 2006 - 2011 (● is the mean of random sample from control batch (bumetanide), ● is the mean of random sample from a batch, n = 45 batches, the error bars, SD, do not show because SD of the batches was < 10%).

RSD 10.89%) with high intra-batch variability. The intra-batch variability (RSD 1.31%) of the blue tablets with the rolex crown logo (batch 38) compared favourably with the intra-batch variability in the mass for the bumetanide control tablets (RSD 0.91%). However, the white tablets with the $E = mc^2$ logo (batch 40) had considerably high intra-batch variability, indicative of poorly made tablets.

3.3.2.2 Diameter

The means of the diameter of round tablets in each of the 41 batches ranged between 7.02 mm to 9.32 mm and the RSD values of the majority (97.6%, n = 40) of the batches of round tablets were under 1%. While the mean of means of diameter of the batches was 8.09 mm, the median was 8.09 mm (RSD 12.0% and RQD 25.83%, Table 3.3, Figure 3.4). The bumetanide control tablets (n = 29) had a mean diameter of 8.12 mm and the RSD was 0.25%.

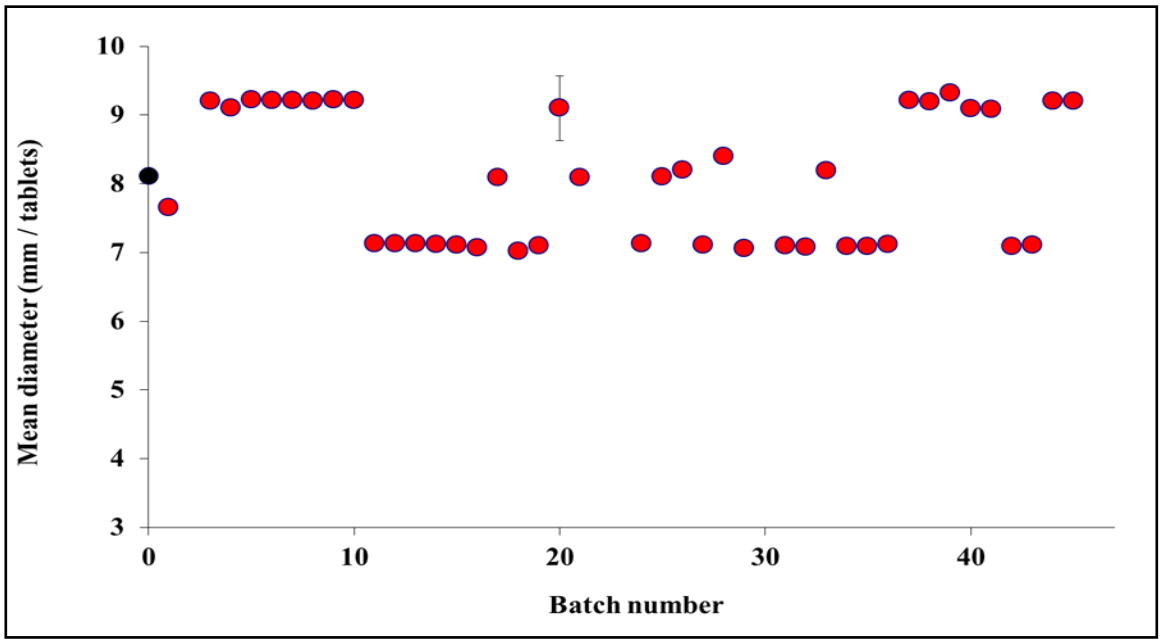


Figure 3.4 Mean diameter (mm) of each batch of round 'ecstasy' tablets over a 5 year period from 2006 - 2011 (● is the mean of random sample from control batch (bumetanide), ● is the mean of random sample from a batch, n = 41 batches, the error bar shown indicate SD, batch 20, SD = 0.47, the rest of the error bars do not show because SD ≤ 0.07).

The diameter of nearly all the examined batches of tablets (97.6%, n = 40 out of 41) and the bumetanide control tablets had satisfied the criterion that tablets with diameter of 12.5 mm can deviate by up to 5% [225]. Only one batch of white tablets with shark logo had tablets with diameters that deviated by more than 5% (Figure 3.4).

3.3.2.3 Thickness

Thickness is another important physical attribute and any variation of this feature on the tablet press may mean a variation in mass or hardness or both. The means of the thickness of tablets in the 45 batches ranged between 3.04 to 6.30 mm and the RSD values of the majority (88.9%, n = 40) of the batches were under 5% (Table 3.3). The mean of means of the thickness of the batches was 3.88 mm and the median was 3.86 mm (RSD 19.1% and RQD 23.1%, Table 3.3. However, the mean thickness of most of the tablets (91.1%, n = 41) was found to vary between 3 and 4.5 mm. For the bumetanide control tablets (n = 29) the mean thickness was 2.73 mm and the RSD was 1.1%.

The thickness of tablets in 33.3% (n = 15) of the batches, did not deviate by more than 5% from the mean. However, more batches (n = 18, 40%) were found to have more than 2 tablets that deviated by more than 5% from the mean thickness.

3.3.2.4 2-D scatter plots of the means of mass, diameter and thickness

The possibility of differentiating or linking batches of 'ecstasy' tablets by the measurable features, mass, diameter and thickness was evaluated by the use of 2-D scatter plots (Figure 3.5 and see Figures 1.1 and 1.2, Appendix 1). Three 2-D scatter plots were used, where the data from 41 batches with round tablets was used for the plots of mass versus diameter and diameter versus thickness, and the data from 45 batches was used for the plot of mass versus thickness.

The batches of tablets were considered linked when the spots, representing the batches on the 2-D scatter plots, were superimposed or touching. The results obtained from the three 2-D scatter plots to discriminate or link batches of tablets were fairly good. Moreover, from the 2-D scatter plots it transpired that 18 batches (in 5 groups of batches: group1 - [5-10], group 2 - [11-13], group 3 - [14-16], group 4 - [29, 36, 42, 43], and group 5 - [44, 45] batch number as in Table 3.2 above) had very similar means for mass, diameter and thickness (Table 3.4).

3.3.2.5 3-D scatter plots of the means of mass, diameter and thickness

The differentiation or linking of the batches of tablets was further evaluated by 3-D scatter plots where the measurable features, mass, diameter and thickness were again used (Figure 3.6 and see Figure 1.3, Appendix 1). Two 3-D scatter plots were used, where data from 41 batches with round tablets was used for the plot of thickness, diameter and mass. To further investigate the possible linkage of the 28 batches of tablets (batches 5-16, 18, 19, 24, 29, 31, 34-38, 40-45) shown in Figure 3.6 as superposed or touching, another 3-D scatter plot of the thickness, diameter and mass was done (see Figure 1.3, Appendix 1).

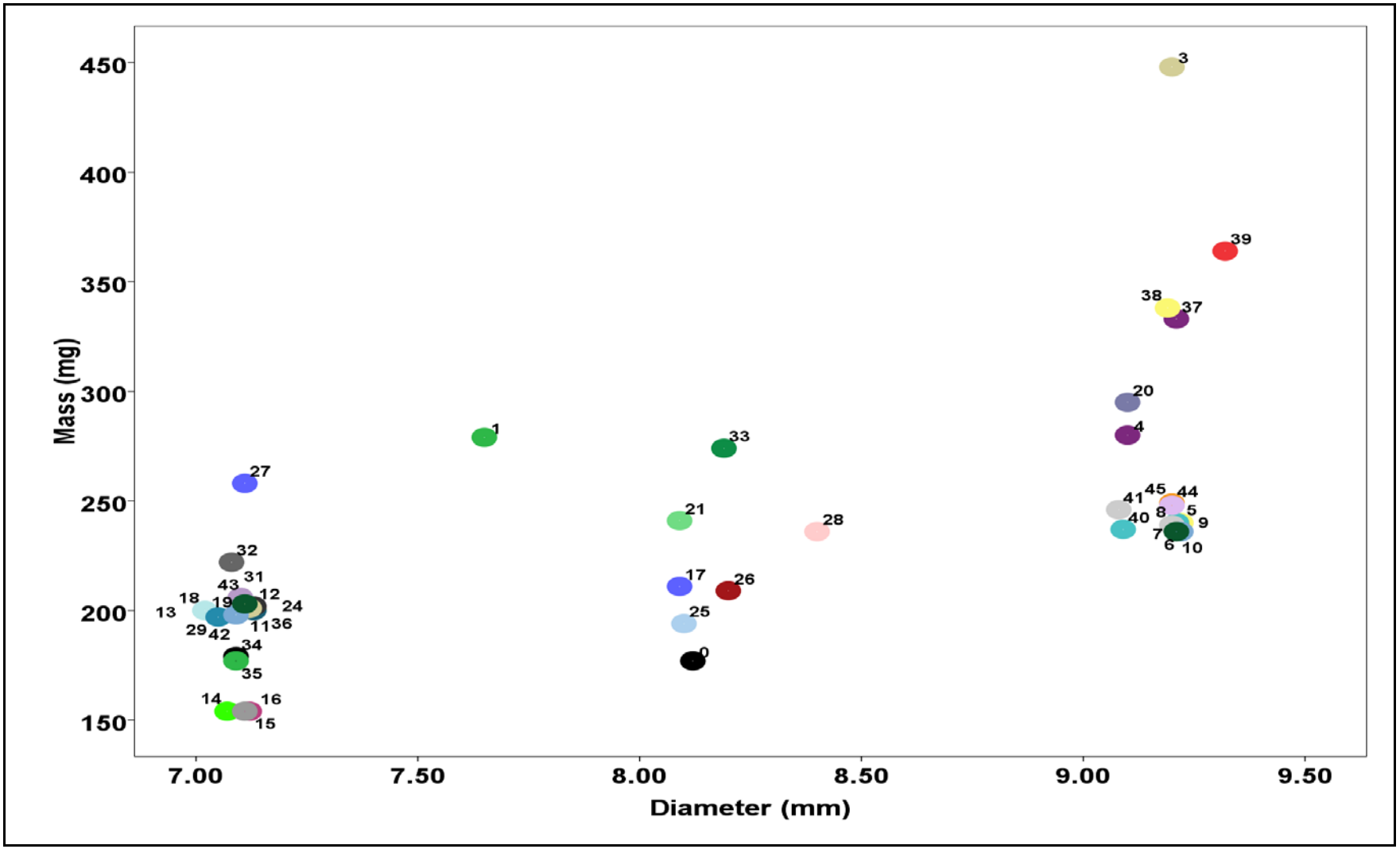


Figure 3.5 2-D scatter plot of the means of mass versus diameter of 41 batches of round 'ecstasy' tablets (the numbers near the coloured spots indicate the batch number as in Table 3.2, batch 0 is the control batch, bumetanide).

Table 3.4 2-D scatter plots results for the batches of tablets (the numbers refer to batch number as given in Table 3.2 above).

2-D Scatter plots	Linked batches	Unlinked batches
Mass vs Diameter (41 batches, Figure 3.5)	[5-10, 44, 45], [11-13, 18, 19, 24, 29, 31, 36, 42, 43], [14-16], [34, 35], [37, 38], [40, 41],	1, 3, 4, 17, 20, 21, 25-28, 32, 33, 39
Mass vs Thickness (45 batches, see Figure 1.1, Appendix 1)	[5-10, 40], [11-13, 18], [14-16], [17,19], [23, 30], [26, 31, 32], [29, 36, 42, 43], [44, 45]	1-4, 20-22, 24, 25, 27, 28, 33-35, 37-39, 41
Diameter vs Thickness (41 batches, see Figure 1.2, Appendix 1)	[5-10], [11-13], [14, 16], [15, 29, 36, 42, 43], [24, 34], [31, 32, 35], [44, 45]	1, 3, 4, 17-21, 25-28, 33, 37-40
Results from the 3 2-D scatter plots (45 batches)	[5-10], [11-13], [14-16], [29, 36, 42, 43], [44, 45]	1-4, 17-28, 30-35, 37-41

The batches of tablets were considered linked when the spots, representing the batches on the 3-D scatter plots, were superimposed or touching. The results obtained from the two 3-D scatter plots to discriminate or link batches of tablets were considered to be fairly good. Moreover, from the 3-D scatter plots it transpired that 20 batches (divided in 4 groups of batches according to similarity in the mean mass, diameter and thickness were: group 1 - [5-10, 40], group 2 - [11-13, 18, 29, 36, 42, 43], group 3 - [14-16], and group 4 - [44, 45], batch number as in Table 3.2 above) had very similar means for mass, diameter and thickness (Table 3.5). The linked batches in the 2-D scatter plots were confirmed by the 3-D scatter plots.

The linkage between the batches was further examined statistically using one-way ANOVA. There was significant similarity in the mass, diameter and thickness of the tablets in batches 5-10, batches 11-13, batches 14-16 (except the diameter) and batches 44 and 45 ($p > 0.05$, one-way ANOVA for nearly all the features). However there was significant difference in the mass, diameter and thickness between the tablets in batch 40

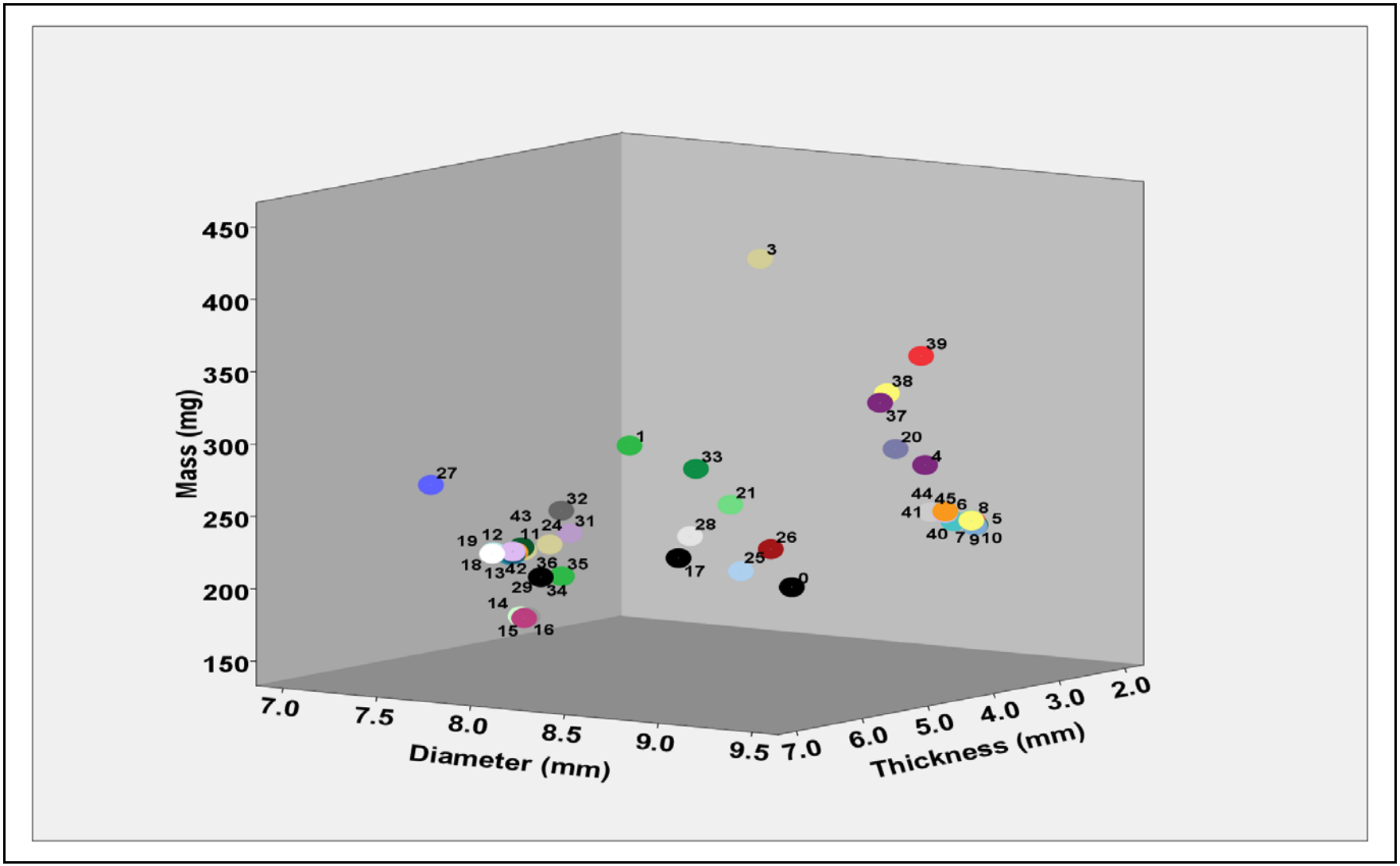


Figure 3.6 3-D scatter plot of the means of thickness, diameter and mass of 41 batches of round 'ecstasy' tablets (the numbers near the coloured spots indicate the batch number as in Table 3.2, batch 0 is the control batch, bumetanide).

and tablets in batches 5-10 and significant difference in tablets in batches 18, 29, 36, 42, 43 and tablets in batches 11-13 ($p < 0.05$, one-way ANOVA).

Table 3.5 3-D scatter plots results for the batches of tablets (the numbers refer to batch number as given in Table 3.2 above)

3-D Scatter plots	Linked batches	Unlinked batches
Thickness, Diameter and Mass (41 batches, Figure 3.6)	[5-10, 40, 41, 44, 45], [11-13, 18, 19, 24, 31, 42, 43], [14-16], [29, 34-36], [37, 38]	1, 3, 4, 17, 20, 21, 25- 28, 32, 33, 39
Mass, Diameter and Thickness (28 batches, see Figure 1.3, Appendix1)	[5-10, 40], [11-13, 18, 29, 36, 42, 43], [14-16], [44, 45]	19, 24, 31, 34, 35, 37, 38, 41
Results from the 2 3-D scatter plots	[5-10, 40], [11-13, 18, 29, 36, 42, 43], [14-16], [44, 45]	1, 3, 4, 17, 19, 20, 21, 24-28, 31-35, 37-39, 41

3.3.3 Other physical characteristics: hardness, friability and disintegration

The results obtained from other physical characterisation which included hardness, friability and disintegration of the 45 batches of 'ecstasy' tablets are given in Table 3.6. These parameters of the 'ecstasy' tablets were compared with the control (bumetanide) tablets, to assess against a known standard.

3.3.3.1 Hardness (mechanical strength)

Although there are no official pharmacopoeial limits for hardness, this feature must be controlled to ensure that the tablet is firm enough to withstand handling without breaking or crumbling and not so hard that the disintegration time is unduly prolonged.

The means of the hardness of the tablets in each of the 45 batches ranged between 37.52 N to 135.29 N and the RSD values of the majority (75.6%, n = 34) of the batches were

Table 3.6 Other measurable physical characteristics, hardness, friability and disintegration of 45 batches of 'ecstasy' tablets from 30 cases.

Batch No.	Tablets	Hardness N	Friability %	Disintegration s	Batch No.	Tablets	Hardness N	Friability %	Disintegration s
0		66.42, 78.41 73.67 (5.96)	0.1	2 ± 1 (50.0)	**23**		48.33, 53.22 50.79 (3.21)	0.4	6 ± 2 (33.3)
1		47.51, 62.69 54.87 (9.00)	0.6	39 ± 11 (28.2)	**24**		47.13, 55.19 50.48 (5.47)	0.5	11 ± 4 (36.4)
2		37.54, 51.89 45.96 (10.49)	0.5	7 ± 2 (28.6)	**25**		48.33, 53.22 50.79 (7.11)	0.4	12 ± 3 (25.0)
3		42.54, 57.12 50.88 (10.34)	0.6	7 ± 2 (28.6)	**26**		33.48, 40.46 37.52 (5.73)	2.0	6 ± 1 (16.7)
4		41.24, 64.29 53.29 (14.52)	0.6	11 ± 3 (27.3)	**27**		72.69, 164.52 104.03 (30.48)	0.4	836 ± 540 (64.6)
5		89.70, 105.81 97.16 (5.03)	0.2	6 ± 3 (50.0)	**28**		36.20, 52.82 42.17 (11.45)	1.6	3 ± 1 (33.3)
6		70.17, 88.40 80.38 (6.83)	0.2	9 ± 5 (55.6)	**29**		80.56, 123.53 105.71 (14.75)	0.2	558 ± 26 (4.66)
7		71.43, 96.68 85.22 (9.61)	0.2	4 ± 7 (175.0)	**30**		82.35, 106.33 93.27 (8.29)	0.1	170 ± 10 (5.88)
8		58.00, 78.60 67.17 (10.45)	0.2	10 ± 6 (60.0)	**31**		72.70, 94.11 84.13 (7.96)	0.5	96 ± 24 (25.0)
9		53.00, 66.24 58.98 (6.75)	0.3	6 ± 4 (66.7)	**32**		50.85, 67.86 59.34 (9.27)	0.1	13 ± 4 (30.8)
10		51.02, 61.77 56.89 (6.24)	0.2	11 ± 4 (36.4)	**33**		44.76, 50.49 47.00 (4.06)	0.9	19 ± 9 (47.4)
11		52.64, 74.60 63.16 (13.27)	3.8	743 ± 478 (64.3)	**34**		60.15, 101.51 79.45 (15.21)	0.2	87 ± 14 (16.1)
12		53. 71, 83.06 68.78 (13.41)	4.4	784 ± 434 (55.4)	**35**		52.82, 79.49 66.19 (13.92)	0.2	77 ± 34 (44.2)
13		48.88, 80.02 58.01 (14.86)	4.0	663 ± 406 (61.2)	**36**		41.17, 63.92 51.70 (16.00)	0.4	124 ± 29 (23.4)
14		44.58, 64.27 53.42 (13.96)	0.8	5 ± 4 (80.0)	**37**		85.40, 106.16 98.34 (6.11)	0.1	147 ± 108 (73.5)
15		41.01, 54.61 47.50 (13.01)	0.6	5 ± 3 (60.0)	**38**		88.44, 123.34 106.09 (9.11)	0.1	258 ± 108 (41.9)
16		41.54, 61.77 48.77 (13.27)	0.3	7 ± 3 (42.9)	**39**		94.16, 124.06 113.07 (7.87)	0.1	160 ± 10 (6.25)
17		53.90, 79.96 64.79 (12.58)	0.6	11 ± 6 (54.5)	**40**		80.93, 132.48 100.85 (17.58)	0.2	313 ± 137 (43.8)
18		51.37, 60.65 57.34 (4.69)	0.5	13 ± 2 (15.4)	**41**		55.68, 131.04 89.57 (24.53)	0.4	242 ± 143 (59.1)
19		49.70, 69.12 61.53 (8.96)	0.7	12 ± 3 (25.0)	**42**		85.40, 162.74 135.29 (19.14)	0.5	722 ± 213 (29.5)
20		43.86, 55.21 49.52 (7.90)	0.5	8 ± 6 (75.0)	**43**		81.64, 129.62 103.85 (13.61)	0.5	436 ± 86 (19.7)
21		42.98, 54.06 47.66 (7.41)	0.7	47 ± 2 (4.26)	**44**		51.38, 116.00 81.76 (21.39)	0.3	242 ± 54 (22.3)
22		64.51, 90.65 77.64 (10.11)	0.3	4 ± 2 (50.0)	**45**		62.23, 96.45 82.89 (12.30)	0.2	224 ± 48 (21.4)

Note: Hardness N – 1st line: the minimum and maximum values of force measured, 2nd line: the mean force and RSD; Friability - % loss in mass of less than 1%; Disintegration (s) – 1st line: the mean and SD, 2nd line: the RSD.

over 7%. While the mean of means of the hardness of the batches was 70.74 N, the median was 63.16 N (RSD 32.80% and RQD 57.89%, Table 3.6, Figure 3.7). The

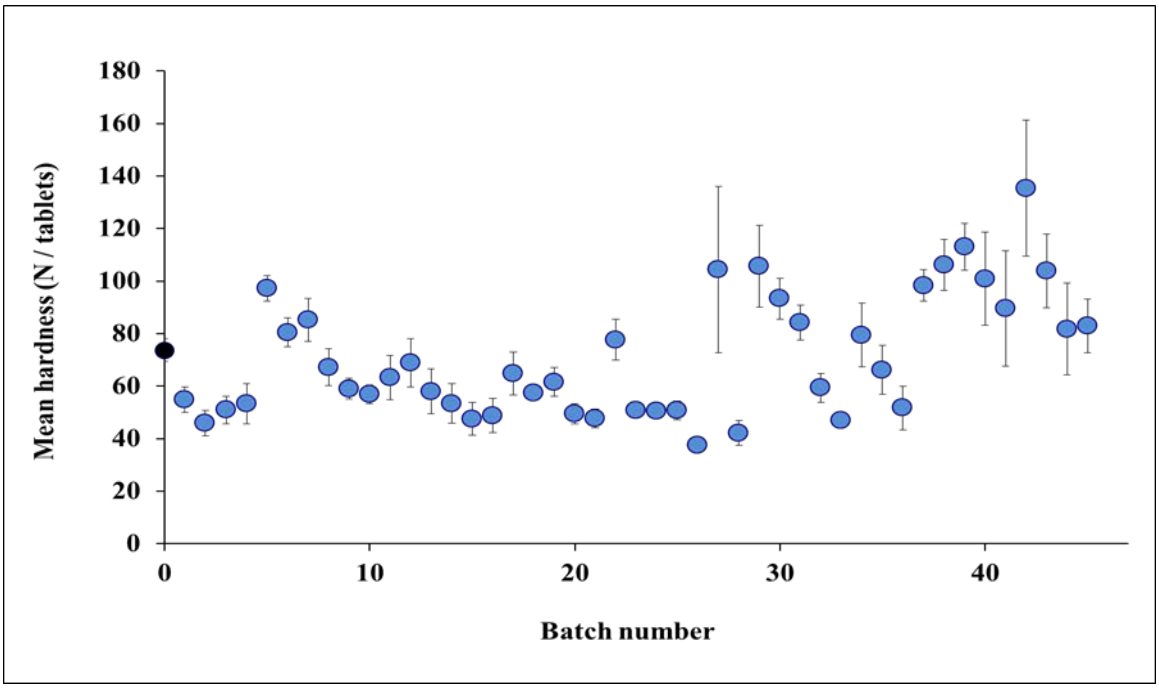


Figure 3.7 Mean hardness (N) of each batch of 'ecstasy' tablets over a 5 year period 2006 - 2011 (● is mean of random sample from control batch (bumetanide), ● is the mean of random sample from a batch, n = 45 batches, the error bars indicate SD).

bumetanide control tablets (n = 29) had a mean hardness of of 73.67 N and RSD of 96%. More than half of the batches (60%, n = 27) had their means within the range for hardness for pharmaceutical tablets (39.22N to 78.45 N). However, it transpired that only 19 batches (42.2%) had all their examined tablets within the hardness range for pharmaceutical tablets, while 51.1% (n = 23) of the batches had 1 tablet or more above the recommended values and 6.7% (n = 3) of the batches had 2 tablets or more below the recommended values. Moreover, batches 27, 29, 39, 41 to 44 had a greater range for hardness than the other batches (Figure 3.7).

3.3.3.2 Friability

The friabilities of the 45 batches of 'ecstasy' tablets ranged between 0.1% to 4.4%. The

mean of the friabilities of the batches was 0.7% and the median was 0.4%. The friabilities of the tablets in the batches were very varied resulting in a very large RSD and RQD (≥ 100%, Table 3.6).

The friability of the bumetanide tablets (0.1%) and the friability of the majority of the batches of 'ecstasy' tablets (89%, n = 40) was found to be less than 1% (Table 3.6). During the friability test the maximum loss in the total mass of the batches tested was not more than 1% and this is normally considered acceptable for pharmaceutically produced tablets.

3.3.3.3 Disintegration

During disintegration tests, which were carried out on all batches of 'ecstasy' tablets, the time required for 6 tablets to disintegrate was measured. The disintegration rate means of the 45 batches of 'ecstasy' tablets ranged between 3 s to 836 s and the RSD values of the

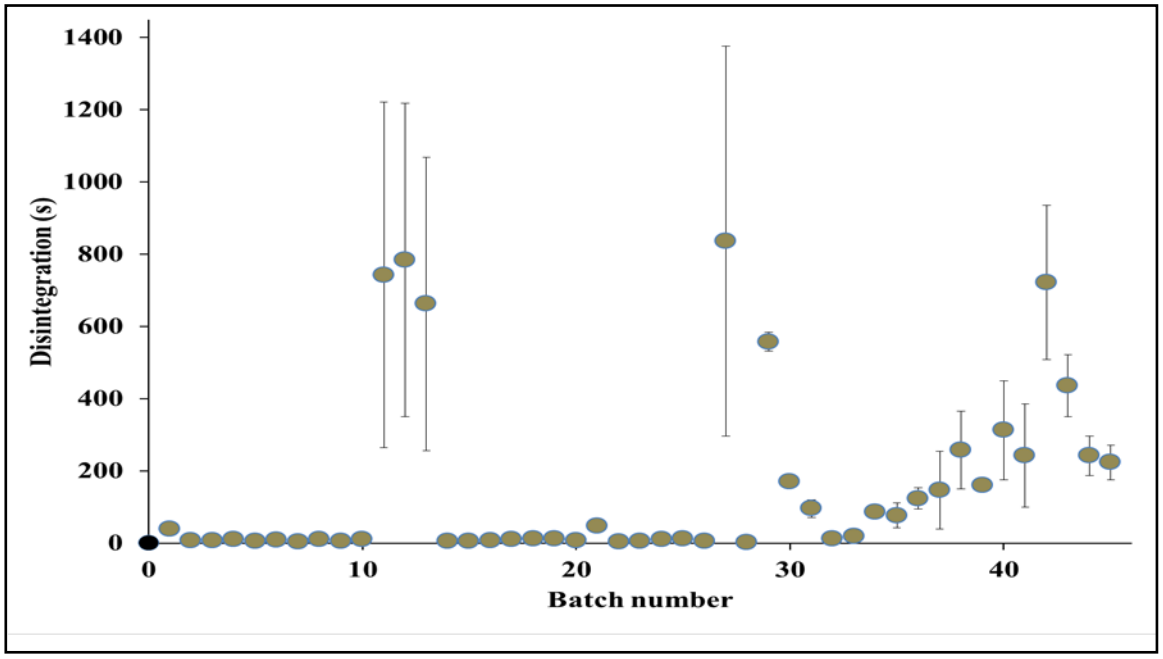


Figure 3.8 Mean disintegration (s) of each batch of 'ecstasy' tablets over a 5 year period 2006 - 2011 (● is mean of random sample from control batch (bumetanide), ● is the mean of random sample from a batch, n = 45 batches, the error bars indicate SD).

majority (91.1%, n = 41) of the batches were over 15%. The mean of the means of disintegrations of the batches was 159 s and the median was 13 s. The disintegration rates of the batches was very varied resulting in a very large RSD and RQD (> 100%) (Table 3.6, Figure 3.8).

More than half (60%, n = 27) of the batches of 'ecstasy' tablets were found to have disintegration rates of less than 60 s (1 min) (bumetanide control tablets – mean disintegration rate was 2 s $\pm$ 1 s, RSD 50%). However, 11.1% (n = 5) of the batches were found to have 2 or more tablets that took more than 900 s (15 min) to disintegrate.

3.3.3.4 2-D scatter plots of friability and means of hardness, disintegration rates and mass

The possibility of differentiating or linking batches of 'ecstasy' tablets by the measurable features, hardness, friability, disintegration rate and mass was evaluated by the use of 2-D scatter plot (Figure 3.9 and see Figures 1.4 to 1.6 in Appendix). Four 2-D scatter plots were done, where the data from 45 batches of 'ecstasy' tablets was used for all the plots. It was noted that the discrimination and linkage of the batches was not as good as the 2-D scatter plots for the mass, diameter and thickness. This was mainly caused by the high RSD values of the batches for the means of hardness and disintegration rates. However, using these plots the linkage between the batches 5-10, 11-13, 14-16 and 44-45 were confirmed (batch number as in Table 3.6 above).

3.3.3.5 3-D scatter plots of the friability and the means of hardness, disintegration rates, mass and diameter

The possibility of differentiating or linking batches of 'ecstasy' tablets by the measurable features, hardness, friability, disintegration rate, mass and diameter was evaluated by the use of 3-D scatter plots (see Figures 1.7 to 1.9, Appendix 1). Three 3-D scatter plots were used, where data from 45 batches of 'ecstasy' tablets were used for the plot of disintegration, hardness and friability and 41 batches with round tablets were used for the plot of diameter, mass and hardness and the plot of friability, diameter and mass. The discrimination and linkage of the batches for the plot of disintegration, hardness and

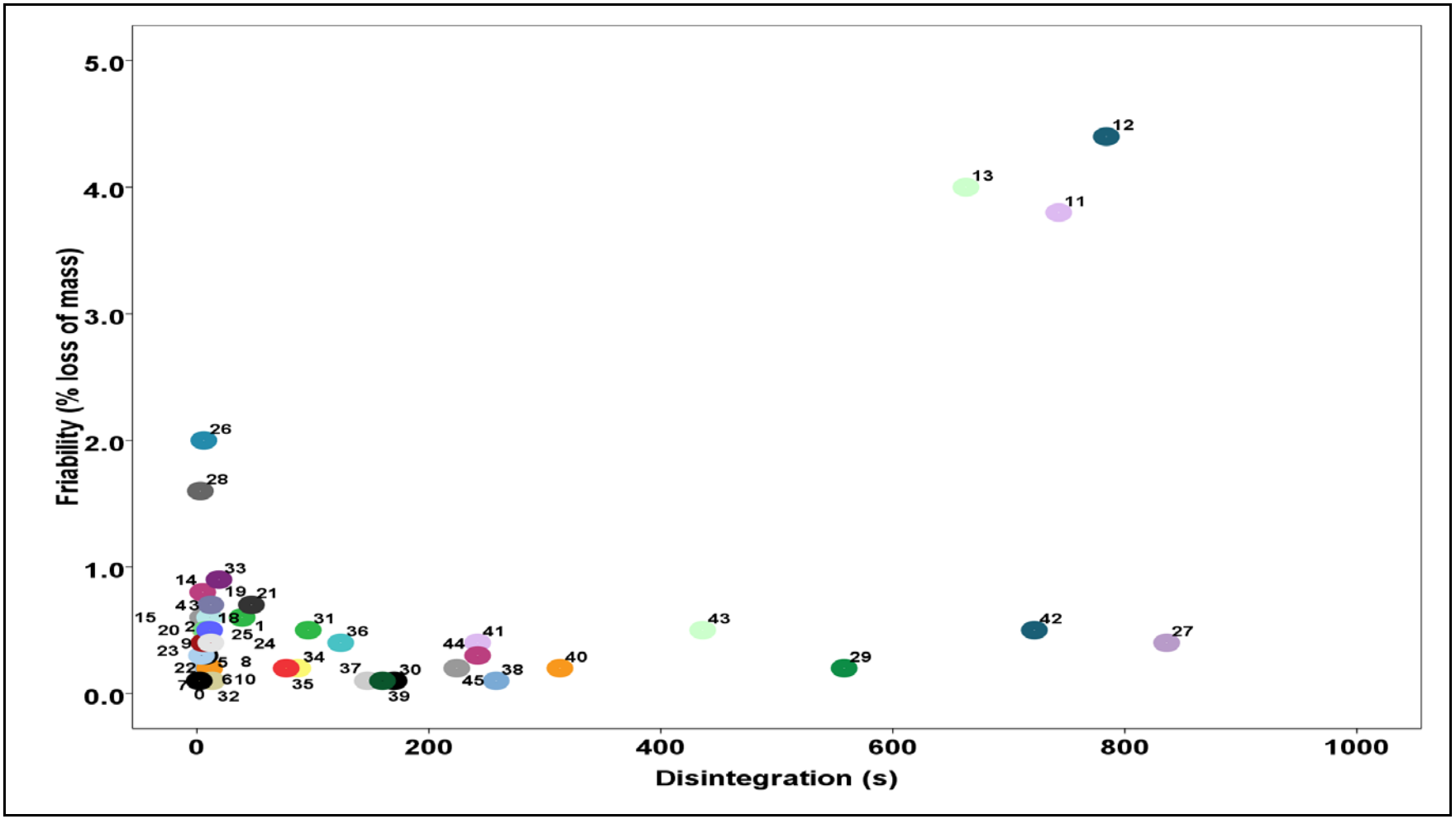


Figure 3.9 2-D scatter plot of the friability versus mean disintegration of 45 batches of 'ecstasy' tablets (the numbers near the spots in the scatter plot indicate the batch number as in Table 3.2, batch 0 is the control batch, bumetanide).

friability and the plot of diameter, mass and hardness was not as good as the 3-D scatter plots for the mass, diameter and thickness. This was mainly caused by the high RSD values of the batches for the means of hardness and disintegration rates. However, using these two plots the linkage between the batches 5-10, 11-13, 14-16 and 44-45 was confirmed and batches 1, 21, 33 and 27 were discriminated (batch number as in Table 3.2 above). Additionally, the plot of friability, diameter and mass was a much better plot than the earlier two 3-D plots to link or discriminate between batches of tablets. Using this plot the linkage between the batches 5-10, 11-13, 14-16 and 44-45 was again confirmed and the batches 1, 20, 21, 26-28, 33 and 39 were discriminated (batch number as in Table 3.2 above). The linkage and differentiation between the batches of tablets were further confirmed statistically using one-way ANOVA.

3.3.4 Quality and linkage

Only four batches of 'ecstasy' tablets satisfied all the six pharmacopoeial and pharmaceutical physical characteristic criteria (mass, diameter, thickness, hardness, friability and disintegration). These batches, which had reached a standard with regards to the studied criteria that would be accepted in pharmaceutics, were batch 18, white tablets (n = 2195) with D&G logo, batch 24, blue tablets (n = 153) with euro logo, batch 32, white tablets (n = 538) with mercedes logo and batch 33, white tablets (n = 837) with Versace logo (total n = 3723). The most poorly made batches of tablets are shown in Table 3.7 and it transpired that batch 40, white tablets (n = 1000) with $E = mc^2$ logo, was the most poorly made batch.

From this study it transpired that there was linkage between fourteen batches of 'ecstasy' tablets from three seizures ($p > 0.05$, one-way ANOVA). The batches of tablets, which had high similarity in their visual and measurable physical features, including RSD and relative quartile deviation (RQD), are shown in Table 3.8. There was even a linkage between the six batches of the blue and green tablets with omega logo (batches 5 to 10). The other thirty one batches of tablets had different physical characteristics ($p < 0.05$, one-way ANOVA).

Table 3.7 Poorly made tablets according to pharmaceutical criteria

Defects of poorly made tablets	Batches
High intra-variation in mass, RSD ranged between 8.27% to 10.89%	40, 44, 45
High intra-variation in thickness, RSD ranged between 6.61% to 9.44%	5, 40, 44, 45
High hardness which ranged between 100.85 N and 135.29 N	27, 38-40, 42, 43
High friability which ranged between 1.6% to 4.4%	11-13, 26, 28

3.3.5 Summary of key findings

Common features of the 45 batches

- The table shape was round (91.1%, n = 41) and with a logo (88.9%, n = 40);
- More than half of the batches of tablets were coloured (57.8%, n = 26);
- The tablets had a mean mass which ranged between 150-250 mg (71.1%, n = 32), a mean diameter which ranged between 7-9 mm (63.4%, n = 26) and a mean thickness which ranged between 3-4 mm (66.7%, n = 30);
- Some of the batches of tablets had a mean mass > 300 mg (n = 6), a mean diameter > 9 mm (n = 16) and a thickness > 5 mm (n = 4);
- The mean hardness was 70.74 N and about one third of the batches of tablets was ≥ 80 N (35.6%, n = 16);
- The friability of most of the tablets was ≤ 0.5% (68.9%, n = 31);

Table 3.8 Batches of 'ecstasy' tablets with similar visual and physical measurable characteristics indicative of a possible linkage between the respective batches.

Batch No.	Tablet	Logo	Year Seizure No.	No. of Tablets	Mass mg	Diameter mm	Thickness mm	Hardness N	Friability %	Disintegration s
5		omega	2007 4	1,021	240 (4.65) 245 (7.16)	9.22 (0.55) 9.20 (0.78)	3.14 (6.66) 3.15 (6.61)	89.70, 105.81 97.16 (5.03)	0.2	6 ± 3 (50.0)
6		omega		1,018	239 (3.32) 241 (6.33)	9.21 (0.47) 9.20 (0.47)	3.09 (3.46) 3.11 (5.76)	70.17, 88.40 80.38 (6.83)	0.2	9 ± 5 (55.6)
7		omega		1.154	240 (3.66) 243 (2.98)	9.21 (0.56) 9.21 (0.68)	3.12 (3.65) 3.14 (3.08)	71.43, 96.68 85.22 (9.61)	0.2	4 ± 7 (175)
8		omega	2007 4	1,029	239 (4.02) 240 (4.70)	9.20 (0.53) 9.19 (0.47)	3.07 (4.76) 3.09 (3.91)	58.00, 78.60 67.17 (10.45)	0.2	10 ± 6 (60.0)
9		omega		1,142	236 (4.21) 237 (4.65)	9.22 (0.45) 9.23 (0.50)	3.09 (3.53) 3.10 (3.27)	53.00, 66.24 58.98 (6.75)	0.3	6 ± 4 (66.7)
10		omega		1,032	236 (4.34) 239 (4.40)	9.21 (0.52) 9.20 (0.65)	3.05 (4.49) 3.10 (4.75)	51.02, 61.77 56.89 (6.24)	0.2	11 ± 4 (36.4)
11		euro	2007 4	1,069	201 (3.22) 200 (3.63)	7.13 (0.10) 7.12 (0.13)	4.11 (3.31) 4.15 (2.13)	52.64, 74.60 63.16 (13.27)	3.8	743 ± 478 (64.3)
12		euro		1,020	202 (4.59) 204 (5.39)	7.13 (0.10) 7.13 (0.08)	4.15 (3.25) 4.17 (3.15)	53. 71, 83.06 68.78 (13.41)	4.4	784 ± 434 (55.4)
13		euro		1,031	200 (4.19) 203 (5.17)	7.13 (0.17) 7.13 (0.18)	4.15 (3.25) 4.17 (2.59)	48.88, 80.02 58.01 (14.86)	4.0	663 ± 406 (61.2)
14		pisces	2007 4	1,114	154 (3.41) 154 (4.55)	7.07 (0.18) 7.07 (0.19)	3.86 (3.36) 3.85 (5.03)	44.58, 64.27 53.42 (13.96)	0.8	5 ± 4 (80.0)
15		pisces		1,344	154 (3.47) 155 (5.66)	7.12 (0.21) 7.12 (0.23)	3.95 (3.80) 3.99 (5.47)	41.01, 54.61 47.50 (13.01)	0.6	5 ± 3 (60.0)
16		pisces		1,379	154 (2.49) 154 (2.60)	7.11 (0.18) 7.11 (0.29)	3.87 (3.88) 3.83 (5.46)	41.54, 61.77 48.77 (13.27)	0.3	7 ± 3 (42.9)
44		lacoste	2011 29	113	249 (8.42) 241 (8.51)	9.20 (0.16) 9.20 (0.16)	3.48 (6.61) 3.39 (7.52)	51.38, 116.00 81.76 (21.39)	0.3	242 ± 54 (22.3)
45		lacoste	2011 30	236	248 (8.27) 244 (7.79)	9.20 (0.15) 9.20 (0.19)	3.48 (7.04) 3.39 (7.76)	62.23, 96.45 82.89 (12.30)	0.2	224 ± 48 (21.4)

Note: Mass, diameter and thickness – 1st line: mean, and RSD, 2nd line: median and RQD, Hardness – 1st line: minimum and maximum values of force measured, 2nd line: mean force and RSD; Disintegration – 1st line: mean and SD, 2nd line: RSD.

- The mean disintegration rate of the batches of tablets was < 900 s (5 min) (84.4%, n = 38).

Discriminating or linking characterising features

- For the visual characteristics the logo (when Europol catalogue was used), shape and breakline were all reliable features to differentiate or link batches of 'ecstasy' tablets. The logo was the most discriminating feature;

- The colour was found to be a very subjective feature and thus fairly reliable when used as a discriminating feature;

- The measurable features mass, diameter and thickness were all reliable features (RSD normally < 5%) and also very good discriminating features;

- The friability and the hardness (RSD normally ≥ 7%) were fairly reliable features but the disintegration rate was a poorly reliable feature (RSD normally ≥ 20%) and thus not recommended as a discriminating feature.

3.4 Discussion

This study focused on the use of the physical characteristics of 'ecstasy' tablets to investigate their possible use for intelligence purposes. A total of 45 batches of tablets (≥ 100 tablets per batch) were seized in Malta over a five year period and the physical characteristics, including the visual features, logo, tablet shape, breakline and colour and the measurable features, mass, diameter, thickness, hardness, friability and disintegration rate were examined. It was shown that the use of some of these physical features could help link or discriminate between batches of tablets. The physical characterisation process of the batches of tablets was carried out in two stages. In the first stage the tablets in the batches were examined for their visual features (logo, shape, breakline and colour). In the second stage the mass, diameter, thickness, hardness, friability and

disintegration rate of the tablets from the batches were measured. The use of the measurable features hardness, friability and the disintegration rate used in this study to differentiate between batches of illicit tablets, as far as is known, is a novel approach and has never been used for forensic intelligence purpose. The data obtained from the physical characterisation of the batches was used to discriminate or link the batches. The linkage of batches by the measurable features mass, diameter, thickness, hardness and disintegration rate was verified by one-way ANOVA. Furthermore, two (2D) and three-dimensional (3D) scatter plots of the measurable features were used to visualize the discrimination or linkage between the different batches. The batches of 'ecstasy' tablets were compared to a test batch of pharmaceutically produced bumetanide tablets.

The possible linking or discrimination of batches of 'ecstasy' tablets normally starts with the examinations of the visual features to assess similarity or not between the batches. The logo, tablet shape and breakline of 'ecstasy' tablets depend on the die and the punches used in the tabletting machine. While the colour of tablets is dependent on the addition of the dye together with other excipients prior to tablet manufacture [196] Using the visual features together (logo, shape, breakline and colour, significant difference between the groups Welch's $F(3,83.30 = 40.40, p < 0.001)$) more than half (68.9%, n = 31) of the examined batches of 'ecstasy' tablets in this study were differentiated from each other. While the majority of tablets had a logo (88.9%, n = 40) and a round shape (91.1%, n = 41), more than half were coloured (55.6%, n = 25). During 2006 - 2009 the trend with regards to breakline on 'ecstasy' tablets was approximately divided between batches with tablets without a breakline and batches with tablets with a breakline on one side running diagonally along the diameter. In 2010 all the examined batches of tablets (n = 7, 4 seizures) had no breakline and in 2011 a new breakline in the form of a cross was noted on one side of three batches of tablets (3 out of 5 seizures). However, some of the batches could not be distinguished from each other because of similar visual features, thus indicating possible linkage. These were batches 5-7, blue tablets and batches 8-10, green tablets both with omega logo, batches 11-13 and 17, white tablets with euro logo, batches 14-16, orange tablets with pisces logo, batches 18 and 29, white tablets with D&G logo, batches 19 and 28, white tablets with heart logo,

batches 24 and 42, blue tablets with euro logo, batches 27 and 34, blue tablets with question mark logo and batches 44-45, pink tablets with lacoste logo.

The mass, diameter and thickness were also used to link or discriminate between batches of 'ecstasy' tablets. These three characteristics are a reflection of the three stage manufacturing process: filling of the die with powder, compression of the powder and ejection of the tablet from the die. The uniformity of mass of tablets in a batch is dependent on the flow properties of the material being tabletted [246]. If the material being tabletted does not flow freely at the required rate then improper filling of the die is possible. Variations in the quantity of powder in each die would result in tablets having different mass and densities [247] such as batches in increasing variation 41, 44, 45 and 40. Hence, large variation in mass of 'ecstasy' tablets does not necessarily mean that tablets were from a different batch. The inadequate quality control during illicit tablet production could cause these variations.

The diameter is the most important physical feature from a forensic point of view because it is produced by the fixed dimensions of the hole of the metallic part in the die of the tabletting machine. This part of the die is never changed by the operator of the tabletting machine. It is also possible to have different diameters within a batch of visually similar tablets, such as batch 20 which consisted of white tablets with shark logo. The high variability in the diameters of illegal tablets could happen when the operator uses more than one punch, for example when using rotary machines. The thickness like the mass of a tablet is determined by the quantity of powder per tablet and the position of the punches in relation to each other during compression.

This study indicated that the values of the mass, diameter and thickness of the tablets sampled from the 45 batches were grouped in three classes: with the mean mass of the batches which ranged between 150 - 300 mg (82.2%, n = 37), the mean diameter of the batches which ranged between 6.5 – 9.5 mm (91.1%, n = 41) and the mean thickness of batches which ranged between 3 – 5 mm (88.9%, n = 40).

The trends in the measurable features, mass diameter and thickness, for the examined batches of 'ecstasy' tablets seized in Malta were compared with trends for the same features for tablets seized in other countries. A study which was conducted in Israel by Levy et al. [139] on 'ecstasy' tablets from 2925 seizure had diameters which ranged between 6.07 – 9.17 mm. Another study by Marquis et al. [181] carried out on 560 samples of 'ecstasy' tablets, which were seized in Finland, Netherlands, Czech Republic, France, Germany, Switzerland and USA, were found to have features which ranged between 150 - 345 mg for mass, 7 – 9 mm for diameter and 3 – 5.4 mm for the thickness. A further study which was conducted in Switzerland by Milliet et al. [214] on 'ecstasy' tablets from 120 seizures had features which ranged between 190 – 308 mg for the mass, 7.1 – 9.5 mm for most of the diameters and 3.2 – 5.8 mm for the thickness. This indicated that the measurable features mass, diameter and thickness of the 'ecstasy' tablets that were seized in Malta were not dissimilar to tablets seized elsewhere in Europe.

The batches of 'ecstasy' tablets that were discriminated by their visual features were further discriminated using two (2D) and three-dimensional (3D) scatter plots. Using the scatter plots for the means of mass, diameter and thickness of the 41 batches of tablets (only round tablets used) 21 batches (51.2%) could be differentiated (batches 1, 3, 4, 17, 19-21, 24-28, 31-35, 37-39 and 41), while 4 groups composed from the remaining 20 batches were linked (group 1 - batches 5-10, 40, group 2 – batches 11-13, 18, 29, 36, 42, 43, group 3 – batches 14-16 and group 4 – batches 44 and 45). The linkage between the batches was further examined statistically using one-way ANOVA. There was significant linkage between batches 5-10, batches 11-13, batches 14-16 (except the diameter) and batches 44 and 45 ($p > 0.05$, one-way ANOVA for nearly all the features). Moreover, batch 40 was differentiated from batches 5-10 and batches 18, 29, 36, 42, 43 were differentiated from each other and from batches 11-13 ($p < 0.05$, one-way ANOVA)

It is sometimes claimed that a link between samples of tablets based on physical characteristics, such as mass, diameter and thickness, does not necessarily mean that the same press was used because it could have been another press with the same settings

[181]. For this reason three other physical features, the hardness and disintegration rates, which are both destructive methods and friability were used to further try to link or discriminate between batches of 'ecstasy' tablets.

It would be difficult to produce tablets having the same hardness, friability and disintegration rates using different tabletting machines because these features depend on the tablet formulation [245] and on the compression force. The hardness of tablets, which is directly related to the compression force used during manufacture, tends to increase with increasing compression force [248]. In normal tablets the friability tends to decrease with increasing compression [248]. With respect to disintegration time this tends to increase linearly with the range of the compression force used. However, it is also claimed that the lubricant used in the tablet production may affect the hardness and the disintegration rate of the tablets [249]. It has been reported that an increase in the mixing time for the lubricant magnesium stearate tended to decrease the hardness but increased the disintegration time of the tablet [249, 250].

The use of the measurable characteristics, the hardness, friability and the disintegration rate to provide data to further link or discriminate batches of illicit tablets as far as is known has never been used for forensic intelligence. This could be because a large number of tablets ($n \approx 50$) would be required to measure these features and also because the measurements of hardness and disintegration rates are destructive.

There was high intra – variation observed within most samples of tablets taken from the batches for the hardness (variation RSD $\geq$ 7%, 77.8%, $n = 35$) and for the disintegration (variation RSD $\geq$ 20%, 84.4% $n = 38$). The discrimination potential for the friability was low when compared with the other five measurable features (mass, diameter, thickness, hardness, disintegration rate). However, although the measurement of these features might not be considered as first priority it was still possible to discriminate between the batches of 'ecstasy' tablets, such as the white tablets with the D&G logo (batches 18 and 29) and the tablets with the heart logo (batches 19 and 28) using these physical features.

The 2-D and 3-D scatter plots of hardness, disintegration rates, friability, mass and diameter helped to confirm the linkage between the batches of 'ecstasy' tablets with similar visual characteristics, the batches 5-7, blue tablets with omega logo, batches 8-10, green tablets with euro logo, batches 11-13, white tablets with euro logos, batches 14-16, orange tablets with pisces logo and batches 44 and 45, pink tablets with lacoste logo. ($p >$ 0.5, both one–way ANOVA, for hardness for most of the samples and for all samples for disintegration rates). The linkage between the batches of 'ecstasy' tablets with similar visual characteristics and similar measurable characteristics could be an indication that the tablets were produced with the same tabletting machines.

The tablets in the forty five batches were assessed for their 'quality' by examining the tablets for defects and their measurable features (mass, diameter, thickness, hardness, friability and disintegration rate) against pharmacopeial and pharmaceutical criteria. Most of the 'ecstasy' tablets in the batches were found to have some form of defect such as sticking, capping, chipping and mottling among others. The defects found on 'ecstasy' tablets were expected because of the illicit manner in which they are produced. When the batches of 'ecstasy' tablets were evaluated for their mass nearly three quarters (73%, n = 33) did comply with the pharmacopeial specification [221], and nearly all batches complied with the diameter criterion [225] (97.6%, n = 40). For thickness only about one-third of the batches complied with the 5% tolerance for pharmaceutically manufactured tablets [225] (33.3%, n = 15). One possibility for the lack of conformity to the criterion for thickness could have been the different pressures of the dies that had been set during the tabletting process [247].

The hardness of tablets is dependent on the mass of the materials and the space between the upper and lower tablet punch at the moment of compression. More than half of the batches (60%, n = 27) had a mean hardness which was within the pharmaceutical tablets criteria values for hardness (39.22 – 78.45 N) [245]. However, there was variability and nearly half of the batches (51.1%, n = 28) had 1 or more of the examined tablets above the recommended pharmaceutical maximum value, while 3 batches had 2 or more tablets below the hardness minimum value for pharmaceutical tablets.

The friability of the majority of batches of tablets (88.9%, n = 40) was found to be in line with pharmaceutically produced tablets (below the 1% cut-off). This indicated that most of the seized batches of tablets were able to withstand handling and transportation during the illicit drug trafficking process.

The absorption of a drug from a solid dosage form, after oral administration, depends on the break-up of the tablet, known as disintegration. The primary role of tablet disintegration is to expose rapidly a large surface area of the drug and thereby facilitate speedy dissolution. More than half (60%, n = 27) of the examined batches of 'ecstasy' tablets were found to have a disintegration rate of less than 60 s (1 min), with the majority (88.9%, n = 40) having a disintegration rate of less than 900 s (15 min). Thus the disintegration rates of most of the examined 'ecstasy' tablets were within the desired time range of 900 s (15 min) for pharmaceutical tablets [225]. The batches of 'ecstasy' tablets which were within the disintegration time range of 900 s (15 min) had mean disintegration rates which ranged between 0.06 seconds to 733 s (12.22 min), while that for the bumetanide tablets was 0.06 seconds.

The good compliance obtained for the pharmacopeial and pharmaceutical criteria for the individual measurable physical features described earlier for the examined batches of 'ecstasy' tablets was not expected (70.3%, n = 187 measurable features from the 45 batches complied to pharmacopeial and pharmaceutical criteria, out of a total of 266). It is clear that there is good expertise available to those producing illicit 'ecstasy' tablets. However, only four batches of tablets were found to satisfy all the criteria laid down by the pharmaceutical industry. These were the white round tablets with D&G logo (batch 18, 2195 tablets, seized in 2007), the blue round tablets with euro logo (batch 24, n = 153 tablets, seized in 2008), the white round tablets with mercedes logo (batch 32, n = 538 tablets, seized in 2008) and the white round tablets with versace logo (batch 33, n = 837 tablets, seized in 2009), which were found not to be linked and hence were manufactured differently by the clandestine tabletting chemist.

It transpired from this part of the study that the small 'ecstasy' market in Malta is

normally characterized by a gradual penetration of tablets with new logos. Nevertheless, this study has also shown that the turnover rate with respect to changes in the physical characteristics, both visual and measurable, of 'ecstasy' tablets seized on different occasions was rather high, such as the colour and logos and difference in the mass, diameter, thickness and hardness of the tablets in the different batches. However, during the period of study batches of 'ecstasy' tablets seized on different occasions did not always have different logos and / or colour, and measurable features. So the ***hypothesis (1)*** that:

> ***The physical state of different batches of 'ecstasy' tablets seized on different occasions in Malta during 2006 – 2011 was significantly different from each other.***

was partially satisfied because 14 batches confiscated during 3 out of the 30 seizures were found to have similar features.

The results from this study have also shown that the physical properties of 'ecstasy' tablets can be used for forensic drug intelligence purposes. The visual features logo, shape, breakline and the measurable features mass, diameter, thickness and friability were found to be very reliable features to be used for characterization to discriminate or link batches of 'ecstasy' tablets. The mass, diameter and thickness were the most discriminating features. Moreover, the two (2D) and three-dimensional (3D) scatter plots were able to show that batches could be linked when they had similar features and discriminated when coming from different batches. A link between batches of tablets based on most of these physical characteristics would make it more difficult to exclude that the same press with the same settings was used to produce the tablets.

Chapter 4

INVESTIGATIONS REGARDING THE STABILITY OF 'ECSTASY' TABLETS

The stability of the physical characteristics (colour, mass, diameter and thickness) of 'ecstasy' tablets is important if these characteristics are to be used to show links between samples of tablets for drug intelligence. In order to explore the stability of the physical features (colour, mass, diameter, thickness, hardness, friability and disintegration) of the seized 'ecstasy' tablets photostability and stability testing were conducted on samples from the 45 batches of tablets to determine how environmental factors, such as UV-visible (VIS) radiation, RH and temperature may alter these features. In addition, the colour and colour difference of 'ecstasy' tablets were evaluated using reflectance spectroscopy, as an added physical discriminator. Storage conditions for 'ecstasy' tablets were recommended based on the findings of these investigations.

4.1 Introduction

In the Netherlands when the tabletting facilities were investigated it was noted that the produced 'ecstasy' tablets had very similar physical features, such as mass, diameter and thickness, but the logos and colours were different [182]. In cases of illegal tabletted drugs, production of the illicit compound and tabletting may not necessarily take place in the same location. Thus similarities in the physical characteristics between different tablet seizures may simply suggest a relationship at the tabletting laboratory [180]. 'Ecstasy' tablets once compressed cannot be changed and their physical characteristics are maintained following trafficking, until seized or consumed. Tablets which have specific characteristics such as mass, diameter and thickness, could be of forensic value if these features are used for intelligence purposes. The physical characteristics of 'ecstasy' tablets could highlight links between batches of tablets with similar physical properties

coming from the same illegal tabletting source, thus connecting separate seizures [214], and discriminating between batches with different external characteristics [181].

Currently in Malta or internationally the comparison of 'ecstasy' tablets from different seizures is mainly based on visual examinations [182] of the physical features such as the logo, colour and shape. The features such as diameter, which is fixed, thickness and mass are also measured [182]. These last two features are dependent on the powder quantity per tablet and the pressure of the punches used during the tablet production [251]. Previous work evaluating the discriminatory effect of both visual and measurable physical features had discarded colour and colour variance of tablets, because it was claimed that these features were not reproducible and that they were operator subjective [181]. During this research study reflectance spectroscopy, was employed as developed by the *Commission International de l' Èclairage* (CIE) to measure the colour of 'ecstasy' tablets. As far as it is known this method has never been used to discriminate between different samples of 'ecstasy' tablets. To further investigate the possible use of colour as a discriminatory physical feature tablets were subjected to photostability stress testing.

As far as we know nothing has been written about the stability of the physical features of 'ecstasy' tablets used for batches discrimination, and whether these measurable characteristics are stable to environmental changes, such as RH and temperature. Studies conducted on legally produced sugar tablet formulation have shown that tablets expanded and became more friable when stored at 71% RH and at 25^{o}C [252]. Similar results were obtained for tablets prepared by direct compression using lactose and microcrystalline cellulose when stored at high RH (85% at 37°C) for short periods of time [253]. Additionally, studies have also shown deterioration in hardness and decrease in friability of pharmaceutical tablets when stored at 75% RH and temperatures of 25 and 40°C respectively [254].

Thus it was hypothesized that 'ecstasy' tablets undergo colorific change with alteration in the light conditions and that measurable features of 'ecstasy' tablet (mass, diameter,

thickness, hardness and friability) would change with alteration to the environmental conditions.

4.2 Setting

'Ecstasy' tablets are thought to be imported to Malta from the Netherlands (N. Harrison, 2011, personal communication, Assistant Commissioner, Malta Police, email, 17 March). It could be speculated that 'ecstasy' tablets produced in the Netherlands may be exposed to a temperate maritime climate which is influenced by the North Sea and Atlantic Ocean. Daytime temperatures in the Netherlands vary from 2-6°C during the moderate winter and 17-20°C in the summer with average daily sunshine that ranges between 2 to 4 hours in winter and to 7 hours in summer. Since the country is small there is little variation in climate from region to region. Furthermore, the average daily RH ranges between 76 to 86% in summer and from 83 to 89% in winter [255]

In Malta 'ecstasy' tablets are subjected to a typical Mediterranean climate which is strongly influenced by the sea. The Islands have a very sunny climate with a daily average of 5 to 6 hours sunshine in mid-winter to more than 12 hours in summer. The summer temperatures ranges between 24°C in May and 32°C and more in August, which is the warmest month, while in winter the average temperature is 16°C. The daily average RH, which is amount of water vapour in the air at a particular temperature, ranges between 67 to 76% in summer and 77 to 81% in winter [255].

The stability of the physical features, colour and the measurable characteristics, of 'ecstasy' tablets, which are used to discriminate between batches of tablets for intelligence purpose, were investigated. Three separate experiments were conducted which included (a) colour, colour variation after photostability stress testing, (b) accelerated storage conditions testing and (c) stability testing at different RHs and temperatures. Prior to the experiments the tablets were all kept in an air conditioned room at RH of 25% and a temperature of 25 $\pm$ 2°C.

During these experiments, which are described in Section 2.2.2 of the "Materials and Methodology", Chapter 2, five different batches of 'ecstasy' tablets (Table 4.1), with different logos and colouration, were used as follows.

4.3 Methodology

Five batches of tablets (batches 5, 8, 11, 14 and 33) plus control tablets (bumetanide) were used to carry out the experiments (Table 4.1).

Table 4.1 Batches of tablets used for the experiments.

Batch No.	Seizure No.	Tablet	Logo	Experiment
5	4		omega	• **Batches** 5, 8, 11 and 14 were used in both **Exps. A and B** • **Exp. A:** photostability stress testing • **Exp. B:** accelerated storage conditions testing;
8			omega	
11			euro	
14			pisces	
control bumetanide tablets	/		/	• **Used in Exp. B:** accelerated storage conditions testing
33	6		versace	• **Exp. C:** stability testing: effect of RH and temperature

Key: Exp. = Experiment

4.3.1 Experiment A: Colour and photostability stress testing

As explained in Section 2.2.2.1, samples of tablets were subjected to photostability stress testing using an adopted ICH method. The samples from batches 5, 8, 11 and 14 were split into two groups of four samples each (Table 4.1). One group of four samples was

exposed to the stress conditions using a cool fluorescent lamp for about 39 days and a UV fluorescent lamp for about 11 hours, while another group of four samples, the control, was covered in foil for the entire experiment to prevent light exposure. At the end of the experiment the colour change in the exposed tablets was compared with control samples. The colour change was evaluated by reflectance spectroscopy using the CIE colour system. The mean CIELAB (CIE $L^*a^*b^*$ - L^* brightness, a^* is the red-green axis, and b^* is the blue-yellow axis) colour results, the ΔE (colour difference between the tablets, control and exposed), the standard deviation (SD) of ΔE.

4.3.2 Experiment B: Accelerated storage conditions

In this experiment, described in Section 2.2.2.2, several observations and measurements were undertaken in order to investigate the stability of tablets when stored for long periods of time up to 180 days. Four different batches of 'ecstasy' tablets (batches 5, 8, 11 and 14) which had different physical features between them (mass, diameter and thickness) were used in this experiment (Table 4.1).

The tablets in the batches and the control bumetanide tablets were kept in storage for 180 days at a temperature of 40 $\pm$ 2°C and a RH of about 75 $\pm$ 5%. The measurable features of the tablets, mass, diameter and thickness, hardness, friability and disintegration rates were measured at the start of the experiments and after 90 and 180 days respectively. Moreover, the mean volume of the tablets was calculated from the obtained data of diameter and thickness using the formula for the volume ($V = \pi r^2 h$, π (pi) = 3.142, r (half the diameter) and h (thickness of tablet).

4.3.3 Experiment C: Stability testing at different RHs and temperatures

In this experiment the mass, diameter, thickness, volume (from data of diameter and thickness), and hardness were calculated for eight different samples of white 'ecstasy' tablets with versace logo (batch 33) (Table 4.1). The tablets were placed under specific conditions as described in methods Section 2.2.2.3. Four samples of tablets (n = 10 each)

were stored at a RH of 33 ± 1% and temperatures of 5, 15, 25 or 35°C respectively. Four other samples of tablets were stored at a RH of 75 ± 1% and temperature of 5, 15, 25 or 40°C respectively. The physical characteristics of each tablet in the eight samples were measured on a weekly basis up to 5 weeks, then at week 8 and subsequently at weeks 10, 12, 14 and 16.

4.4 Results

4.4.1 Colour and colour variation and photostability stress testing

The colour change for the samples of exposed 'ecstasy' tablets from batches 5, 8 11 and 14, that were subjected to photostability stress testing, and control tablets (covered with foil) are described in Table 4.2. The change in the absorbance ≈ 0.6 between the two actinomeric solutions ('exposed' and 'covered') indicated that the length of light exposure was sufficient and in accordance with ICH recommendations for photostability stress testing [227].

When the individual (CIE) L^* (brightness), a^* (red / green) and b^* (yellow / blue) colour space values of the 'exposed' green tablets with omega logo (batch 8) were compared with the 'control' tablets no statistical difference in colour change was found between the tablets. However, there was a significant increase ($p < 0.001$) in the b^* values for the 'exposed' white tablets with the euro logo (batch 11) when compared with 'control' tablets. This was due to a statistically significant difference in yellowness in the exposed tablets (increase) for batch 11. There was also a significant increase ($p = 0.009$) in the L^* values for the 'exposed' blue tablets with omega logo (batch 5) when compared with 'control' tablets, indicating a significant change in brightness and also a significant increase ($p < 0.001$) in the a^* values indicating a decrease in greenness. Furthermore, for the 'exposed' orange tablets with pisces logo (batch 14) when compared with 'control' tablets, there was a significant decrease in redness ($p < 0.001$, decrease in a^* values), and a significant decrease in yellowness ($p < 0.001$, decrease in b^* values) as shown in Table 4.2.

Table 4.2 CIELAB colour space mean values (*L - brightness, *a** - red / green and *b** - yellow / blue) for the 8 samples of tablets (4 samples exposed and 4 samples covered with foil - controls) and change in colour of exposed tablets.**

Batch Number	Tablets	Tablets Under Control Conditions	Tablets Exposed to Stress Conditions	*p* value	ΔE*	SD of ΔE	Colour Differences [a] [229]
5		(L*) 72.6 (0.48) (a*) -3.3 (0.61) (b*) -8.0 (4.26)	(L*) 72.9 (0.4) (a*) -3.0 (3.7) (b*)-7.8 (5.4)	**0.009** **<0.001** 0.128	0.42	0.27	very slight
8		(L*) 80.7 (1.07) (a*) -5.6 (7.0) (b*) 17.5 (2.1)	(L*) 80.8 (0.9) (a*) -5.8 (4.5) (b*) 17.6 (1.9)	0.685 0.966 0.548	0.54	0.70	slight
11		(L*) 86.9 (0.6) (a*) 1.5 (9.9) (b*) 8.5 (2.5)	(L*) 87.1 (1.0) (a*) 1.5 (11.0) (b*) 8.7 (4.0)	0.403 0.111 **<0.001**	0.42	0.49	very slight
14		(L*) 83.3 (0.2) (a*) 9.7 (1.3) (b*) 37.8 (1.6)	(L*) 83.6 (0.8) (a*) 9.0 (8.3) (b*) 32.8 (6.2)	0.053 **<0.001** **<0.001**	5.06	1.98	very obvious

Note: The numbers in bold text indicate statistical significance.
[a] Colour difference is subjective.

Photostability testing: key findings

- Significant increase in the brightness and significant decrease in the greenness of the tablets in batch 5;
- No change in batch 8;
- Significant increase in the yellowness of the exposed tablets in batch 11;
- Significant decrease in the redness and yellowness of the tablets in batch 14, which was very obvious change.

The tablets with 'very obvious' colour change were the range coloured tablets from batch 14 (E^* = 5.06, SD 1.98) (Table 4.2). The colour of these tablets had faded as indicated by the significant decrease in a^* ($p < 0.001$) and b* ($p < 0.001$).

4.4.2 Accelerated storage conditions testing: effects on physical features

The purpose of accelerated storage conditions testing was to investigate changes in the measurable physical characteristic, mass, diameter, thickness, volume, hardness, friability and disintegration rates of 'ecstasy' tablets. Changes in these characteristics could affect the comparative examinations of 'ecstasy' tablets for intelligence purposes.

4.4.2.1 Baseline measurements of tablet samples used for accelerated storage at day one

Table 4.3 gives an overview of the measurable features of the tablets (mass, diameter and thickness), at day one (baseline). At day one the mean mass of the tablets in these four 'ecstasy' batches varied between 154 - 240 mg, while the mean diameter and thickness of

Table 4.3 Properties of the 4 samples (n = 80 tablets each) of 'ecstasy' tablets from different batches including the control tablets used in the experiments on day one (baseline).

Tablets	Mass (mg)	Diameter (mm)	Thickness (mm)	Hardness [a] (N)	Friability (%)	Disintegration (s) ± SD
 Control	177 (0.91)	8.1 (0.25)	2.7 (1.10)	a = 76.7 (7.96) b = 66.4 c = 87.0	0.1	2 ± 1 (50.0)
 batch 5	240 (4.65)	9.2 (0.55)	3.1 (6.66)	a = 97.2 (5.03) b = 89.7 c = 105.8	0.2	6 ± 3 (50.0)
 batch 8	239 (4.02)	9.2 (0.53)	3.1 (4.76)	a = 67.2 (10.45) b = 58.0 c = 78.6	0.2	10 ± 6 (60.0)
 batch 11	201 (3.22)	7.1 (0.14)	4.1 (3.42)	a = 63.2 (13.27) b = 52.6 c = 74.6	3.8 chipped, capped	743 ± 478 (64.3)
 batch 14	154 (3.41)	7.1 (0.18)	3.9 (3.36)	a = 53.4 (13.96) b = 44.6 c = 64.3	0.8	5 ± 4 (80.0)

Note: The numbers in parenthesis are the RSD
[a] Hardness (mechanical strength) – (a) refers to the mean value (b) refers to the minimum value of force measured and (c) refers to the maximum value of force measure.

the tablets varied between 7.1 - 9.2 and 3.1 – 4.1 mm respectively. The control tablets bumetanide had a mean mass of 177 (RSD 0.91%) mg, while the mean diameter and thickness were 8.1 (RSD 0.25%) mm and 2.7 (RSD 1.10%) mm respectively. The mean hardness of all the tablet samples varied between 53.4 – 97.2 N. The mean hardness of the control tablets was 76.7 N.

At baseline the friability of the batches of 'ecstasy' tablets, with the exception of batch 11 (white tablets with the euro logo), was below 1%. The tablets from batch 11 were found to chip (breaking away of the surface of the tablet) and cap and so the friability test had to be excluded. At baseline, the disintegration time of the 'ecstasy' tablets was found to be less than 780 s (13 min), which was better than the standard required for licit uncoated tablets. The friability of the control bumetanide tablets was less than 1% and the disintegration time about 2 s. (Table 4.3).

It was also determined that the major excipients of the tablets used for the accelerated storage conditions testing were sorbitol for the blue and green tablets with omega logo (batches 5 and 8) and orange tablets with pisces logo (batch 14) and lactose for the white tablets with euro logo (batch 11) (see Section 5.3.4).

4.4.2.2 Effects on 'ecstasy' tablets and control bumetanide tablets after 90 and 180 days of accelerated storage conditions

Tablet mass

There was a significant decrease in the mean mass of the tablets from batch 14, of 6 mg (p = 0.004, one-way ANOVA), after 90 days. The mass of the tablets from batch 14 could not be measured after 180 days because the tablets had disintegrated. The mean mass of control tablets and the tablets from batch 11 after 180 days respectively remained unchanged compared to initial measurements. The decrease of 3.5 mg in the mean mass of the tablets from both batches 5 and 8 at the end of the experiment was not statistically significant (Table 4.4).

Table 4.4 Change in the mass of 'ecstasy' tablets after 90 and 180 days following accelerated storage conditions.

Tablets	Mass (mg)				
	Day 1	**Day 90**	**Change observed after 90 days**	**Day 180**	**Change observed after 180 days**
blue omega logo batch 5	242 (3.40) [a]	241 (3.43)	decrease - 1 mg from day 1 ($p > 0.05$)	239 (3.53)	decrease – 3 mg from day 1 ($p > 0.05$) decrease – 2 mg from day 90 ($p > 0.05$)
green omega logo batch 8	239 (4.39)	236 (4.34)	decrease - 3 mg from day 1 ($p > 0.05$)	235 (4.29)	decrease – 4 mg from day 1 ($p > 0.05$) decrease – 1 mg from day 90 ($p > 0.05$)
white euro logo batch 11	199 (4.69)	199 (4.63)	no change ($p > 0.05$)	199 (4.62)	no change ($p > 0.05$)
orange pisces logo batch 14	156 (2.76)	150 (5.45)	decrease – 6 mg from day 1 **($p = 0.001$)**	tablets disintegrated	
bumetanide control	176 (1.71)	176 (1.71)	no change ($p > 0.05$)	176 (1.71)	no change from days 1 and 90 ($p > 0.05$)

[a] ***Mean mass followed by relative standard deviation (RSD) in parenthesis.***

Tablets diameter

There was a significant increase ($p \leq 0.001$, one-way ANOVA) in the mean diameter of batch 5 and 8 and 11 after 90 days and 180 days respectively (Table 4.5 below). The friability of the 'ecstasy' tablets from batch 14 had increased so much after 90 days that their diameter could not be measured because the tablets had begun to disintegrate. There was no statistically significant change in the mean diameter of the control tablets.

Table 4.5 Change in the diameter of 'ecstasy' tablets after 90 and 180 days following accelerated storage conditions.

Tablets	Diameter (mm)				
	Day 1	**Day 90**	**Change observed after 90 days**	**Day 180**	**Change observed after 180 days**
blue omega logo batch 5	9.23 (0.43) [a]	9.52 (0.53)	increase - 0.29 mm from day 1 **($p < 0.001$)**	9.58 (0.52)	increase - 0.35 mm from day 1 **($p < 0.001$)** increase - 0.06 mm from day 90 **($p < 0.001$)**
green omega logo batch 8	9.21 (0.87)	9.41 (1.06)	increase - 0.20 mm from day 1 **($p < 0.001$)**	9.49 (0.95)	increase - 0.28 mm from day 1 **($p < 0.001$)** increase - 0.08 mm from day 90 **($p = 0.001$)**
white euro logo batch 11	7.13 (0.14)	7.19 (0.14)	increase - 0.06 mm from day 1 **($p < 0.001$)**	7.23 (0.14)	increase - 0.10 mm from day 1 **($p < 0.001$)** increase - 0.04 mm from day 90 **($p < 0.001$)**
orange pisces logo batch 14	7.07 (0.14)	tablets very friable			
bumetanide control	8.16 (0.12)	8.16 (0.12)	no change ($p > 0.05$)	8.17 (0.12)	slight change from days 1 and 90 ($p > 0.05$)

[a] ***Mean diameter followed by relative standard deviation (RSD) in parenthesis.***

Tablets thickness

There was significant increase in the mean thickness ($p < 0.001$, one-way ANOVA) of batch 5 and 8 respectively after 90 days. Moreover, there was also significant increase in mean thickness ($p < 0.001$, one-way ANOVA) of batch 5 and 8 respectively from day 90

to day 180 and batch 11 of (p = 0.004, one-way ANOVA) after 180 days (Table 4.6 below). The thickness of the tablets from batch 14 could not be measured because of the high increase in the friability of the tablets. There was no statistically significant difference in the mean thickness of the control tablets.

Table 4.6 Change in the thickness of 'ecstasy' tablets after 90 and 180 days following accelerated storage conditions.

Tablets	Thickness (mm)				
	Day 1	**Day 90**	**Change observed after 90 days**	**Day 180**	**Change observed after 180 days**
blue omega logo batch 5	3.14 (4.78) [a]	3.54 (4.24)	increase - 0.40 mm from day 1 **($p < 0.001$)**	3.68 (3.53)	increase - 0.54 mm from day 1 **($p < 0.001$)** increase - 0.14 mm from day 90 **($p = 0.002$)**
green omega logo batch 8	3.07 (4.56)	3.27 (3.98)	increase - 0.20 mm from day 1 **($p < 0.001$)**	3.44 (3.78)	increase - 0.37 mm from day 1 **($p < 0.001$)** increase - 0.17 mm from day 90 **($p < 0.001$)**
white euro logo batch 11	4.10 (4.15)	4.16 (4.09)	increase - 0.06 mm from day 1 (p = 0.345)	4.25 (3.77)	increase - 0.15 mm from day 1 **($p = 0.004$)** increase - 0.09 mm from day 90 (p = 0.148)
orange pisces logo batch 14	3.86 (3.37)	tablets very friable			
bumetanide control	2.77 (1.81)	2.78 (1.80)	increase - 0.01 ($p > 0.05$)	2.78 (1.80)	as day 90 ($p > 0.05$)

[a] ***Mean thickness followed by relative standard deviation (RSD) in parenthesis.***

Tablets volume

There was a significant increase ($p \leq 0.013$, one-way ANOVA) in the mean volume, of batch 5 and 8 and 11 after 90 days and 180 days respectively (Table 4.7 below). There

was no statistically significant change in the mean volume of the control tablets. The friability of the tablets from batch 14 had increased so much after 90 days that their volume could not be measured because the tablets had begun to disintegrate.

Table 4.7 Change in the volume of 'ecstasy' tablets after 90 and 180 days following accelerated storage conditions.

Tablets	Volume (mm^3)				
	Day 1	**Day 90**	**Change observed after 90 days**	**Day 180**	**Change observed after 180 days**
blue omega logo batch 5	210.22 (4.98) [a]	252.04 (4.52)	increase - 41.82 mm^3 from day 1 **($p < 0.001$)**	264.93 (3.44)	increase - 54.71 mm^3 from day 1 **($p < 0.001$)** increase - 12.89 mm^3 from day 90 **($p < 0.001$)**
green omega logo batch 8	204.85 (4.92)	227.48 (4.55)	increase - 22.63 mm^3 from day 1 **($p < 0.001$)**	243.34 (3.80)	increase - 38.49 mm^3 from day 1 **($p < 0.001$)** increase - 15.86 mm^3 from day 90 **($p < 0.001$)**
white euro logo batch 11	163.76 (4.22)	169.22 (4.11)	Increase - 5.46 mm^3 from day 1 **($p = 0.009$)**	174.47 (3.86)	increase - 10.71 mm^3 from day 1 **($p < 0.001$)** increase - 5.25 mm^3 from day 90 **($p = 0.013$)**
orange pisces logo batch 14	146.33 (3.60)	very friable			
bumetanide control	145.11 (1.61)	145.34 (1.87)	increase - 0.23 mm^3 from day 1 ($p > 0.05$)	145.48 (1.91)	increase - 0.37 mm^3 from day 1 ($p > 0.05$) increase - 0.14 mm^3 from day 90 ($p > 0.05$)

[a] ***Mean volume followed by relative standard deviation (RSD) in parenthesis.***

Tablets hardness

After storage for 90 days there was a significant decrease ($p < 0.001$, one-way ANOVA), in the mean hardness of the tablets from batch 5 (97.16 N, RSD 5.03% to 39.28 N, RSD

7.33%) and a significant decrease in the hardness of the tablets from batch 11 after 180 days (63.16 N, RSD 8.38% to 42.85 N, 6.45%). Only 5 out of 10 tablets, selected at random from batch 5, could be examined after 90 days because the rest were very friable. After 90 days no tests could be conducted on the tablets from batch 8 because these were too friable, while the tablets from batch 14 started to disintegrate (Table 4.8).

Table 4.8 Change in the hardness of 'ecstasy' tablets after 90 and 180 days following accelerated storage conditions.

Tablets	Hardness (N)				
	Day 1	**Day 90**	**Change observed after 90 days**	**Day 180**	**Change observed after 180 days**
blue omega logo batch 5	97.16 (5.03) [a]	39.28 (7.33)	decrease - 57.88 from day 1 **($p < 0.001$)**	very friable	
green omega logo batch 8	67.17 (10.45)	very friable			
white euro logo batch 11	63.16 (13.27)	50.13 (11.69)	decrease - 13.03 from day 1 **($p = 0.001$)**	42.84 (16.25)	decrease - 20.32 from day 1 **($p = 0.001$)** decrease - 7.29 from day 90 ($p = 0.075$)
orange pisces logo batch 14	48.77 (13.96)	very friable / disintegrated			
bumetanide control	76.66 (9.67)	80.98 (8.76)	increase - 4.32 from day 1 ($p > 0.05$)	79.83 (9.76)	increase - 3.17 from day 1 ($p > 0.05$) decrease - 1.15 from day 90 ($p > 0.05$)

[a] ***Mean thickness followed by relative standard deviation (RSD) in parenthesis.***

Friability and disintegration of tablets

The friability of the tablets from batches 5 and 8 was found to have increased by 1.4%

and 10.5% respectively after 90 days and by 4.0% and 21.3% respectively after 180 days, whereas the friability of the control tablets remained unchanged (0.1%) at 180 days. Some of the tablets from batch 11 were found to chip (breaking away of the surface of the tablet) and cap (complete or partial separation of the upper or lower surface), hence the high friability at days 1 and 180. The tablets from batch 14 did not survive the accelerated stress test. After 90 days the tablets were very friable and disintegrated after

Table 4.9 Friability and disintegration of 'ecstasy' tablets after 90 and 180 days following accelerated storage conditions.

Tablets	Friability (%) and Disintegration (s) ± SD and (RSD)		
	Day 1	Day 90	Day 180
blue omega logo batch 5	0.2 6 ± 3 (50.0)	1.6 1 ± 1 (100)	4.2 2 ± 2 (100)
green omega logo batch 8	0.2 10 ± 6 (60.0)	10.7 1 ± 1 (100)	21.5 3 ± 2 (66.7)
white euro logo batch 11	3.8 (some tablets chipped / capped) 752 ± 480 (63.8)	0.73 524 ± 548 (104.6)	2.6 (some tablets chipped / capped) 771 ± 121 (15.7)
orange pisces logo batch 14	0.3 7 ± 3 (42.9)	100 /	disintegrated
bumetanide control	0.1 2 ± 1 (50.0)	0 64 ± 5 (7.8)	0.1 62 ± 12 (19.4)

180 days (Table 4.9, Figures 4.1 and 4.2), hence the friability and disintegration tests were not done. The mean disintegration rates of the tablets from batches 5 and 8 had significantly decreased (p = 0.002, one-way ANOVA and Welch test respectively) after

90 days and thereafter there was no significant change ($p > 0.05$, one-way ANOVA and Welch test respectively) in the disintegration in both batches till day 180 (Table 4.9). The mean disintegration rates of the tablets from batch 11 did not change significantly

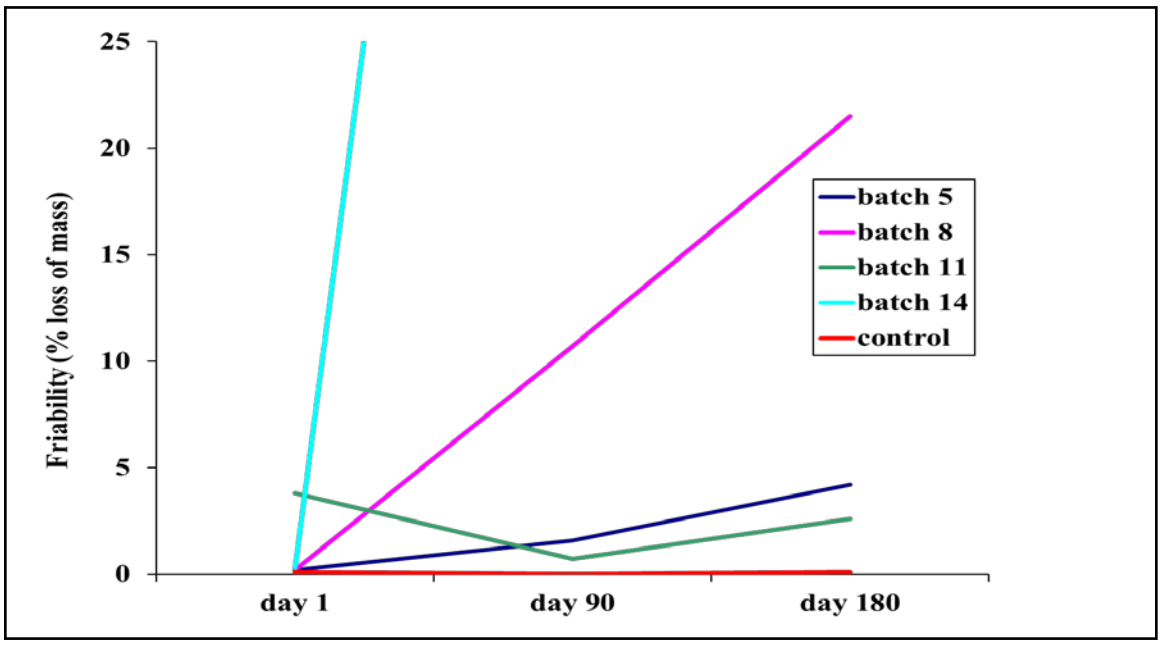


Figure 4.1 Change in the friability of 'ecstasy' tablets after 90 and 180 days of accelerated storage conditions at 75% RH and 40°C. Batch 14 at day 90 friability was 100%.

after 90 and 180 days respectively ($p > 0.05$, Welch test) (Table 4.9). Moreover, the mean disintegration rates of the control tablets had significantly increased ($p < 0.001$, Welch test) after 90 days and thereafter there was no significant change in the disintegration till day 180 ($p > 0.05$, Welch test).

Figure 4.2 The orange tablets with pisces logo (batch 14) after 90 and 180 days of accelerated storage conditions, stored at 75% RH and 40°C.

Accelerated storage: key findings

Mass

- Significant decrease in the mean mass of tablets from batch 14 after storage of 90 days at 75% RH and 40°C;
- At 180 days no change in batch 11 and non significant decrease in the mean mass of batches 5 and 8 and disintegration of batch 14;

Diameter

- Significant increase in the mean diameter of tablets from batches 5, 8 and 11 after storage of 90 days at 75% RH and 40°C;

Thickness

- Significant increase in the mean thickness of tablets from batches 5, 8 and 11 after storage of 180 days at 75% RH and 40°C;

Volume

- Significant increase in the mean volume of tablets from batches 5, 8 and 11 after storage of 180 days at 75% RH and 40°C;

Hardness

- Significant decrease in the mean hardness of tablets from batches 5 and 11 after 90 days storage at 75% RH and 40°C;
- Batch 8 and 14 too friable to conduct test at day 90 and batch 5 to conduct test at day 180;

Friability

- increase in the friability of the all the batches;

Disintegration rate

- Significant decrease in the mean disintegration rates of tablets from batches 5 and 8 at day 90 at 75% RH and 40°C;
- There was no significant change in the disintegration rates of tablets from batch 11 after 90 and 180 days respectively;
- Batch 14 to friable to conduct test.

4.4.3 Stability testing: effect of RH and temperatures on the physical features of 'ecstasy' tablets

4.4.3.1 Baseline measurements of tablet samples used for stability experiment

Table 4.10 shows the physical properties of the 'ecstasy' tablets from batch 33 (with versace logo) that were used for the stability experiment. At baseline, the hardness and the friability of the tablets were within the acceptable limits for pharmaceutical tablets.

Table 4.10 Baseline properties of the 80 'ecstasy' tablets which were then divided into 8 samples of 10 tablets each for subsequent experiments.

	**White coloured 'ecstasy' tablets with Versace logo – batch 33**
Mass (mg)	Mean = 275 (2.39) Range = from 263 to 286
Diameter (mm)	Mean = 8.20 (0.37) Range = from 8.15 to 8.30
Thickness (mm)	Mean = 4.40 (1.57) Range = from 4.25 to 4.57
Hardness (N) [a]	(i) 47.00 (4.06%), (ii) 44.76, (iii) 50.49,
Friability (%)	0.89

Note: the numbers in parenthesis are the relative standard deviation (RSD).
[a](i) mean value (RSD), (ii) minimum value of force measured and (iii) maximum value of force measured.

At baseline, the frequency distribution of the mass showed a strong bimodal distribution (Figure 4.3 below). This indicated the presence of two distinct tablet populations within the sample of 80 tablets which had been taken at random from one batch. It was also

determined that the major excipient of the white tablets with versace logo was lactose (Section 5.3.4).

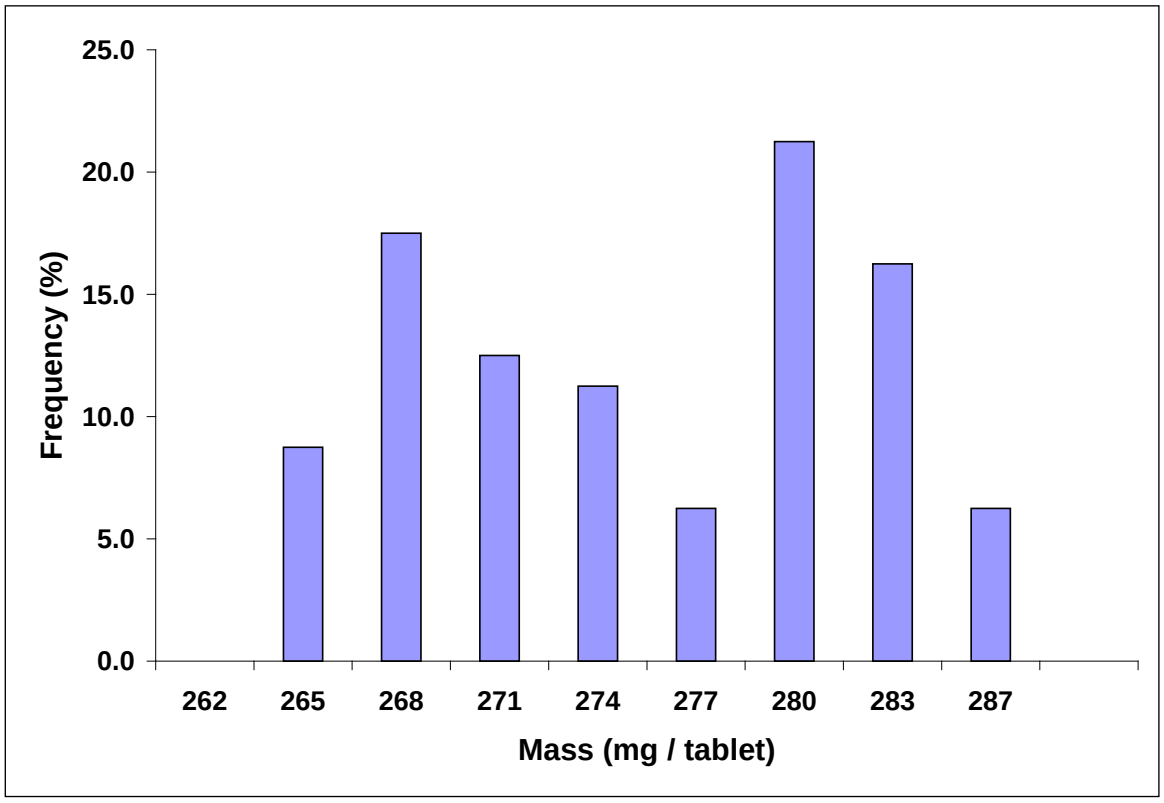


Figure 4.3 Frequency distribution of the mass of 'ecstasy' tablets with versace logo from batch 33 used during the stability study (Week 0).

4.4.3.2 Temperature and RH effects

This section gives the results on the effects of RHs (33-75%) at different temperatures (5, 15, 25, 35 and 40°C) on the physical features (mass, diameter, thickness, volume, hardness and friability) of the examined 'ecstasy' tablets.

Tablets mass

Although there was no significant statistical difference between the mean mass of the tablets stored at 33 and 75% RH for the separate temperatures between weeks 0 and 1, it

was noted that for the tablets stored at 33% RH there was a slight decrease in mean mass at 5, 25 and 35°C and a slight increase for all samples stored at 75% RH (Table 4.11).

Table 4.11 Mean mass (mg) of 'ecstasy' tablets at week 0 and week 1 when exposed at 5, 15, 25 and 35°C and 33% RH and at 5, 15, 25 and 40°C and 75% RH (Number in the parenthesis is SD).

Week	33% RH			
	Temperatures (°C)			
	5	15	25	35
0	270.5 (6.62)	275.1 (7.16)	275.9 (7.02)	277.6 (6.79)
1	269.6 (6.50)	276.0 (7.45)	273.4 (6.65)	270.3 (6.48)
p – value one-way ANOVA	0.763	0.786	0.424	0.920

Week	75% RH			
	Temperatures (°C)			
	5	15	25	40
0	275.2 (6.18)	274.5 (5.68)	273.5 (7.44)	274.0 (5.33)
1	278.5 (6.50)	278.7 (5.85)	275.1 (7.78)	274.6 (5.56)
p – value one- way ANOVA	0.851	0.621	0.997	1.000

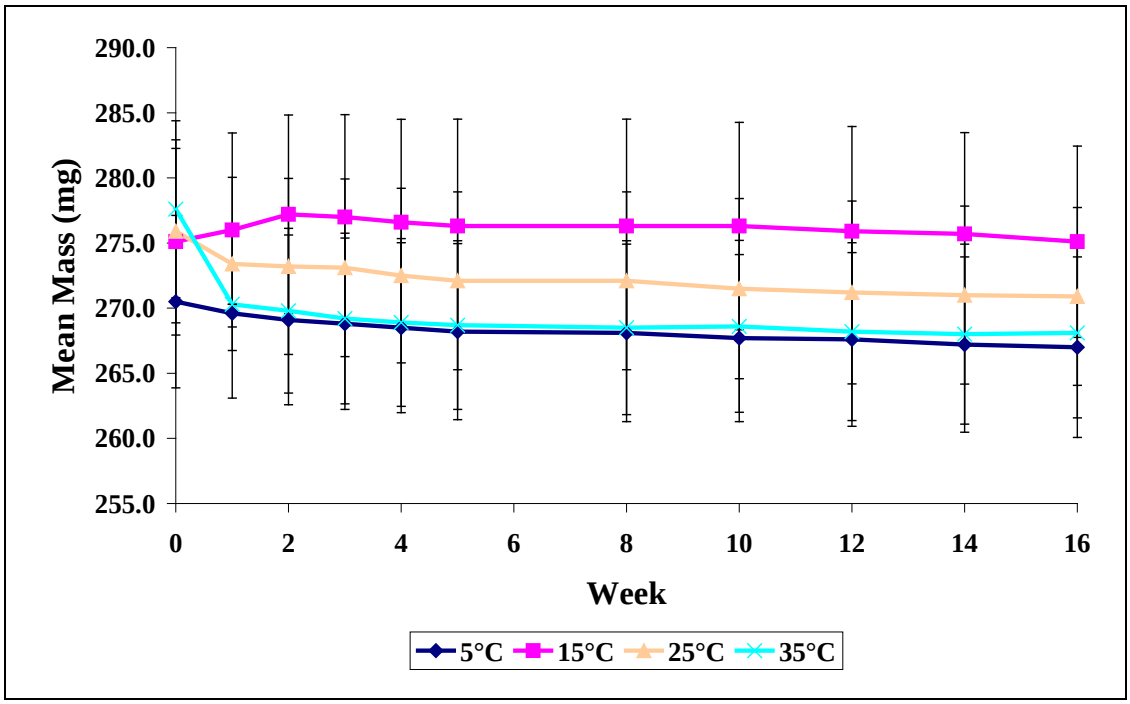
Based on the trends shown in Figure 4.4 (A) there was no change in the mean mass of tablets at 15°C at 33% RH over 16 weeks. There was a slight decrease in the mass at temperatures of 5 and 25°C and a decrease in the mean mass at 35°C after 16 weeks when stored at 33% RH.

At the start of the experiment (week 0) there was no difference ($F(3,36) = 1.936$, p =

0.141, one-way ANOVA) between the mean mass of the tablets in the 4 separate samples which were then stored at 33% RH and separate temperatures (5, 15, 25 and 35°C). Investigating the weekly change in the mean mass of the 4 separate samples of tablets under the different storage conditions there was significant difference ($p < 0.05$, one-way ANOVA) between the mean mass of the tablets for weeks 2 to 4, 10 and 14. However, no significant difference ($p > 0.05$, one-way ANOVA) existed between the mean mass of tablets in the 4 separate samples for the other weeks (see Table 1.1, Appendix 2). Moreover, there was no significant change in the mean mass ($p > 0.05$, one-way ANOVA) of 3 out of 4 separate samples of tablets that were stored at 33% RH and 5, 15, and 25°C respectively, after 16 weeks when compared to the mean mass of the separate samples at the start of the experiment (week 0). For the samples of tablets stored at 33% RH and 35°C there was a significant decrease ($p = 0.06$, one-way ANOVA) of 3.5% in the mean mass of tablets after 16 weeks when compared with the mean mass at week 0 (Figure 4.4, B).

For the tablets stored at 75% RH and all temperatures (5, 15, 25 and 40°C) at week 0 there was no difference ($F(3,36) = 0.137$, $p = 0.938$, one-way ANOVA) between the mean mass of the samples. Additionally, there was no significant difference when comparing the mean mass of the 4 separate samples of tablets stored at 75% RH and all temperature for each week, from week 1 to 16 ($p > 0.05$, one-way ANOVA) (see Table 1.1, Appendix 2). For the tablets stored at 75% RH at 5 and 15°C respectively there was a slight increase in mass after 1 week, and thereafter there was a slight decrease till week 16. For the tablets stored at 25 and 40°C there was a slight continuous decrease throughout the 16 weeks (Figure 4.5, A). There was no significant change in the mean mass of the 4 separate samples of tablets when stored at 75% RH and all temperature after 16 weeks when compared to the mean mass of the separate samples at week 0 ($p > 0.05$, one-way ANOVA) (Figure 4.5, see Table 1.1, Appendix 2). Additionally, there was no significant difference when comparing the mean mass of the 4 separate samples of tablets stored at 75% RH at all temperature (5, 15, 25 and 40°C), for each week, starting from week 1 to week 16 ($p > 0.05$, one-way ANOVA) (see Table 1.1, Appendix 2).

A



B

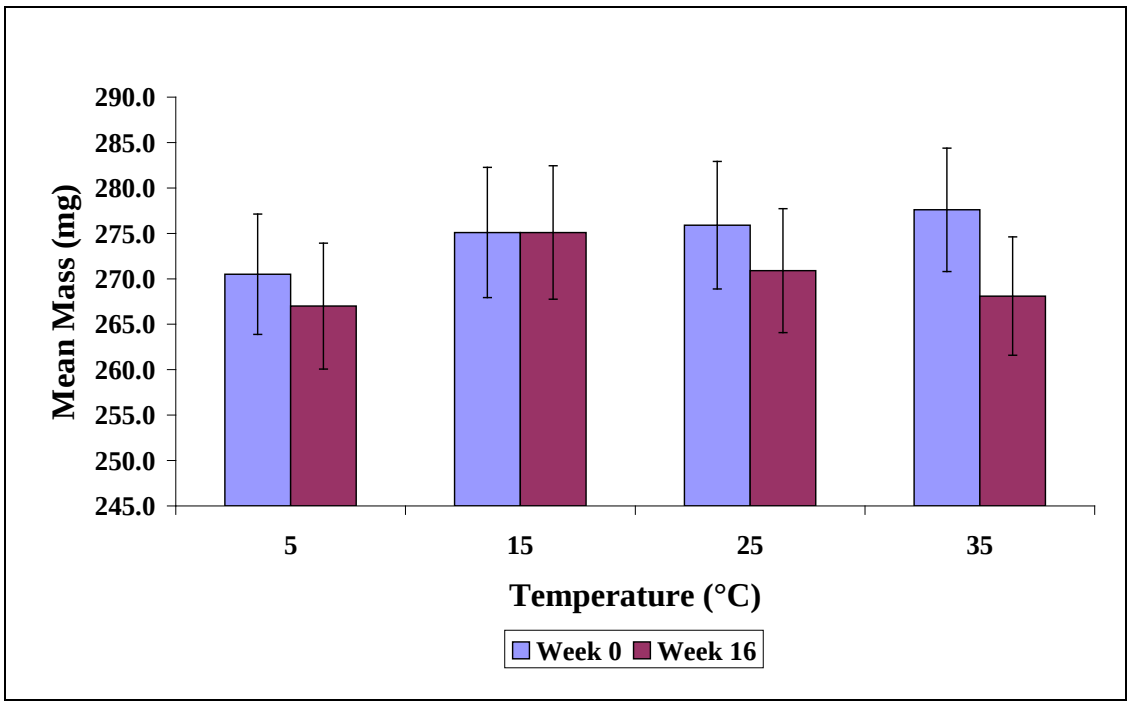
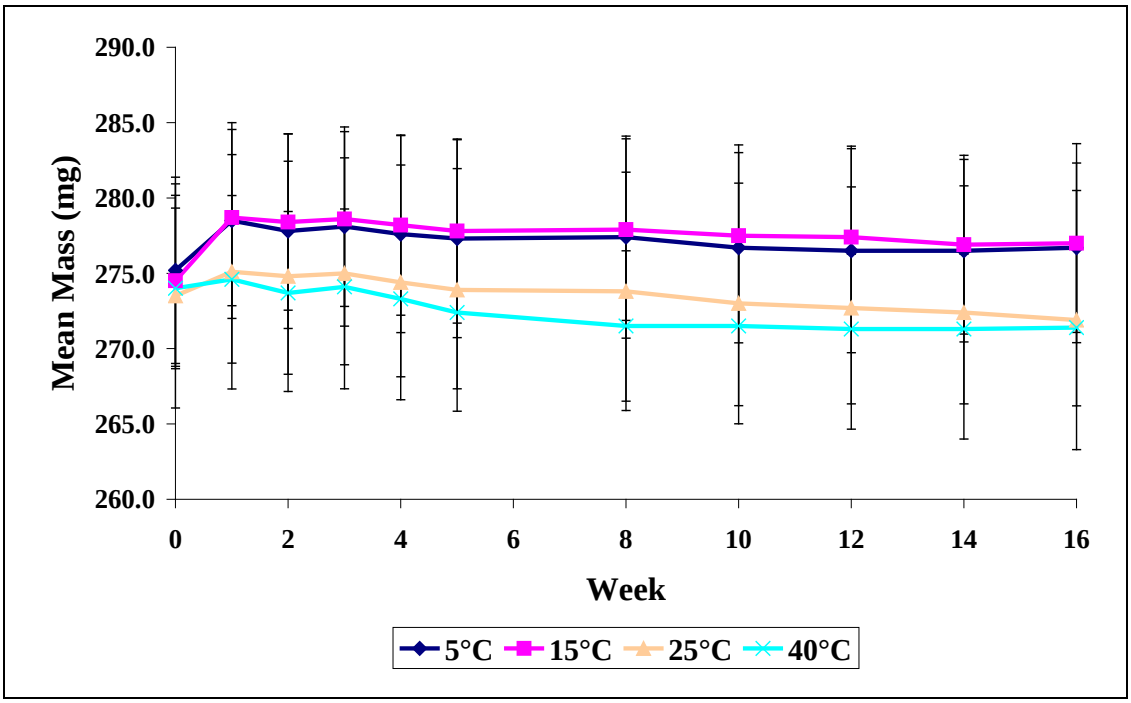


Figure 4.4 A: Mean mass (mg) of 'ecstasy' tablets exposed at 5, 15, 25 and 35°C and 33% RH between 0 and 16 weeks (Errors bars overlap). B: Comparison of mean mass at weeks 0 and 16. (Error bars in A and B represent the SD of each mean).

A



B

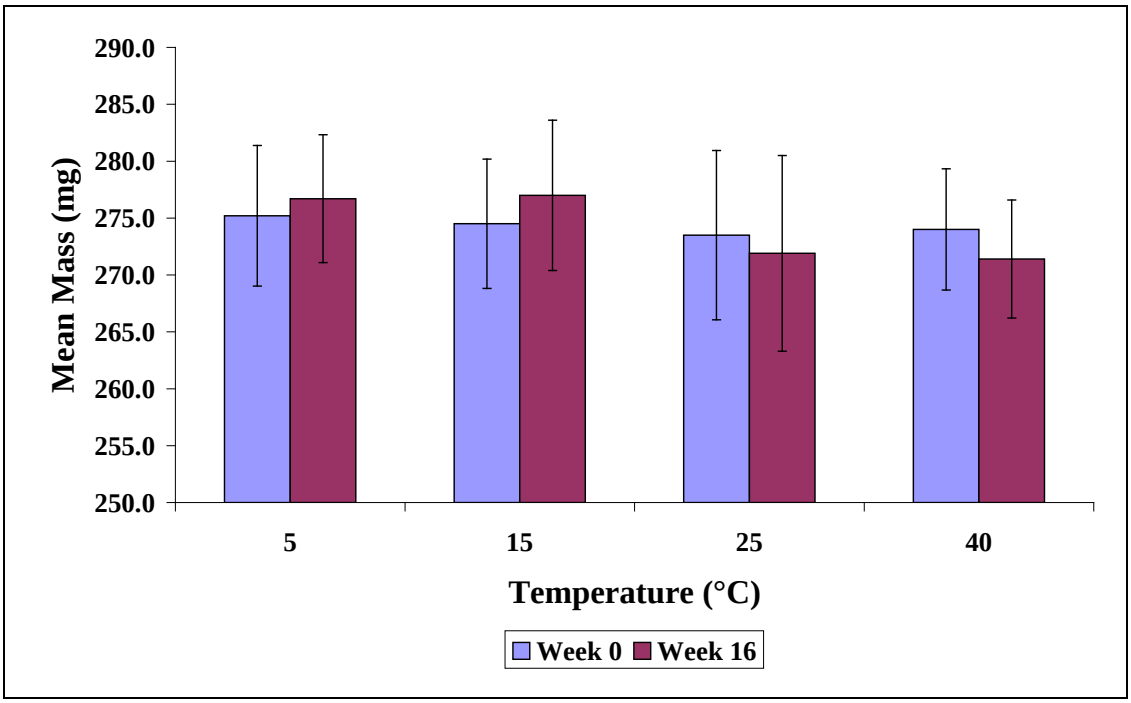


Figure 4.5 A: Mean mass (mg) of 'ecstasy' tablets exposed at 5, 15, 25 and 40°C and 75% RH between 0 and 16 weeks (Errors bars overlap). B: Comparison of mean mass at weeks 0 and 16. (Error bars in A and B represent the SD of each mean).

At week 16 there was no significant difference between the mean mass of the two samples of tablets stored at 33 and 75% RH at 15°C and the two samples at 25°C respectively ($p > 0.05$, one-way ANOVA) (Figure 4.6). There was also no statistically significant difference in the mean mass of the two separate samples of tablets stored at 33 and 75% RHs and at separate temperatures of 35 and 40°C ($p > 0.05$, one-way ANOVA). However, at week 16 there was a significant increase ($p = 0.008$, one-way ANOVA) in the mean mass of tablets stored at 75% RH and temperature of 5°C when compared with the sample of tablets stored at 33% RH and similar temperature (Figure 4.6, see Table 1.1, Appendix 2).

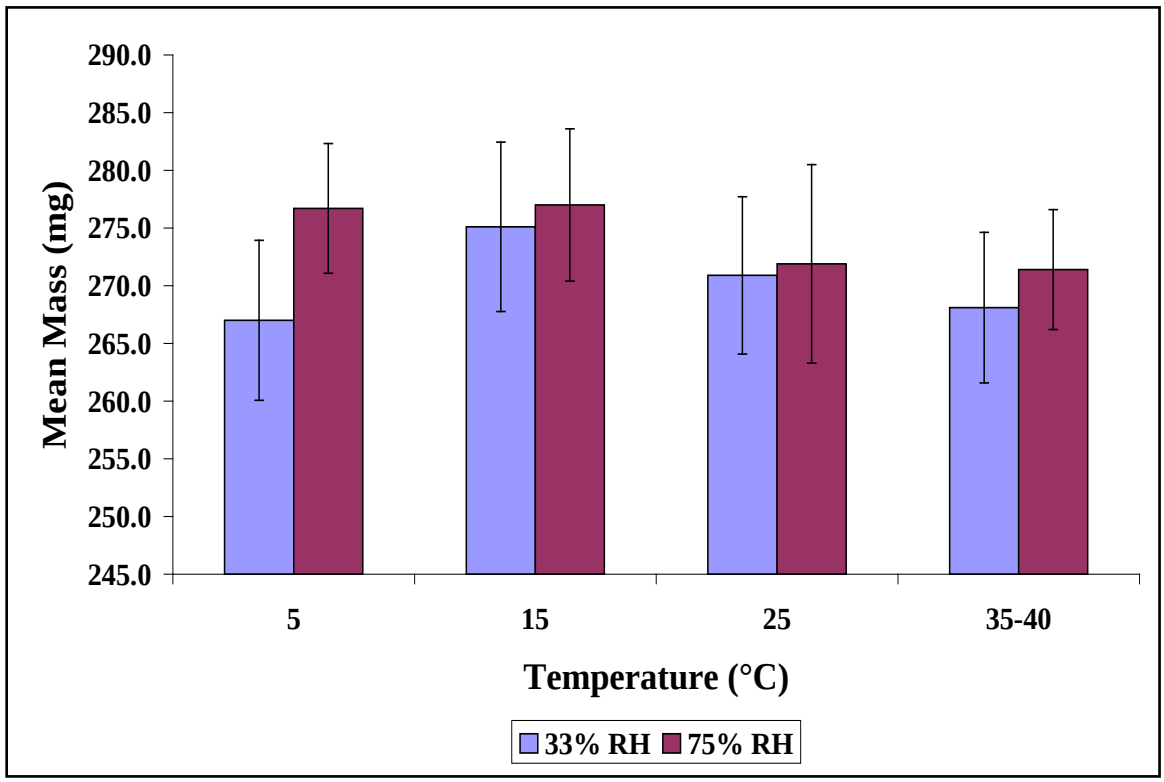


Figure 4.6 Mean mass (mg) of 'ecstasy' tablets exposed at 5, 15, 25 and 35°C and 33% RH and at 5, 15, 25 and 40°C and 75% RH at week 16 (Error bars represent the SD of each mean).

If only the RH is considered there was no significant difference between the mean mass of the total tablets (n = 40, refers to 4 samples with 10 tablets each) in the 4 samples that were stored at 33% RH for all weeks (F (10, 429) = 1.130, $p = 0.338$, one-way ANOVA).

Moreover, there was no significant difference between the mean mass of the total tablets (n = 40) in the 4 samples that were stored at 75% RH for all weeks (F (10, 429) = 0.796, p = 0.632, one-way ANOVA).

The tablets stored at 75% RH had a significantly greater mass (p < 0.05, one-way ANOVA) on week by week basis when compared to tablets stored at 33% RH, regardless of temperature (Figure 4.7, see Table 1.2, Appendix 2). Moreover, while there was a non significant decrease (1.64%, p = 0.2 one-way ANOVA) in the mean mass of the total tablets (n = 40) stored at RH 33% after 16 weeks, the mean mass of the total tablets (n = 40) stored at RH 75% remained largely unchanged as week 0 after 16 weeks (Figure 4.7, see Table 1.2, Appendix 2).

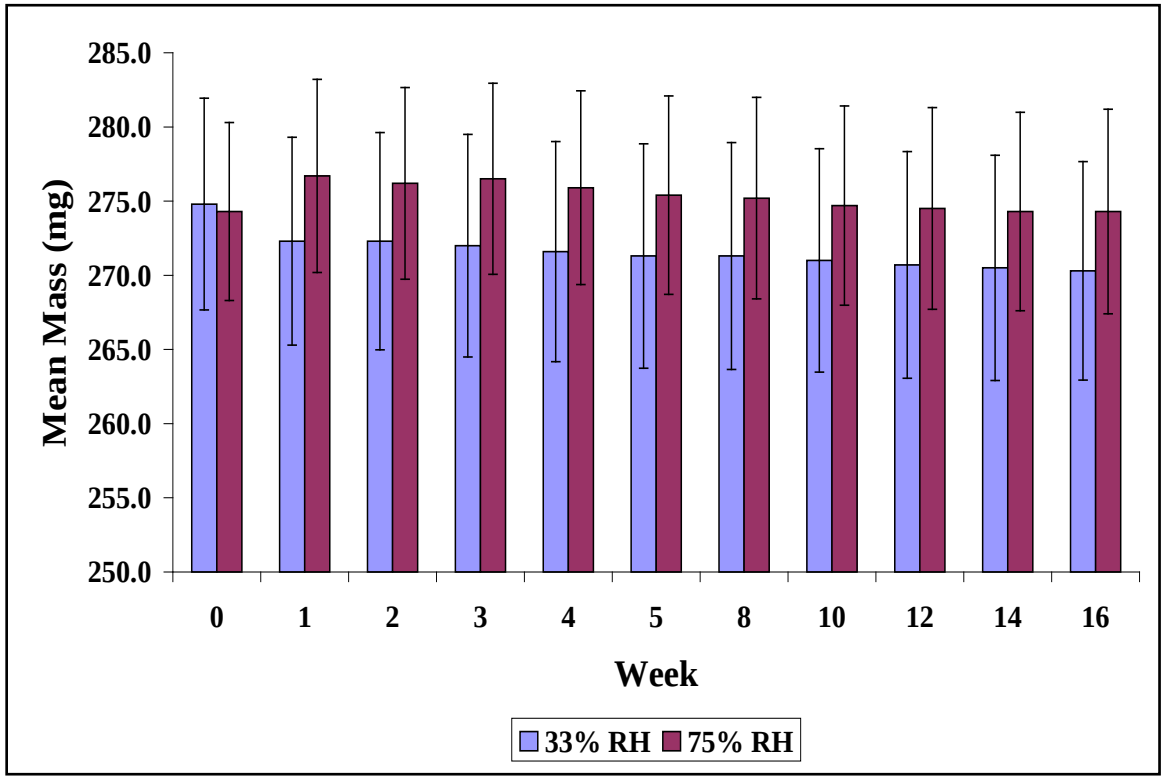


Figure 4.7 Mean mass (mg) of total 'ecstasy' tablets (n = 40, 10 tablets in each of the 4 samples) exposed at 33% RH and mean mass of total 'ecstasy' tablets (n = 40) exposed at 75% RH between 0 and 16 weeks (Error bars represent the SD of each mean).

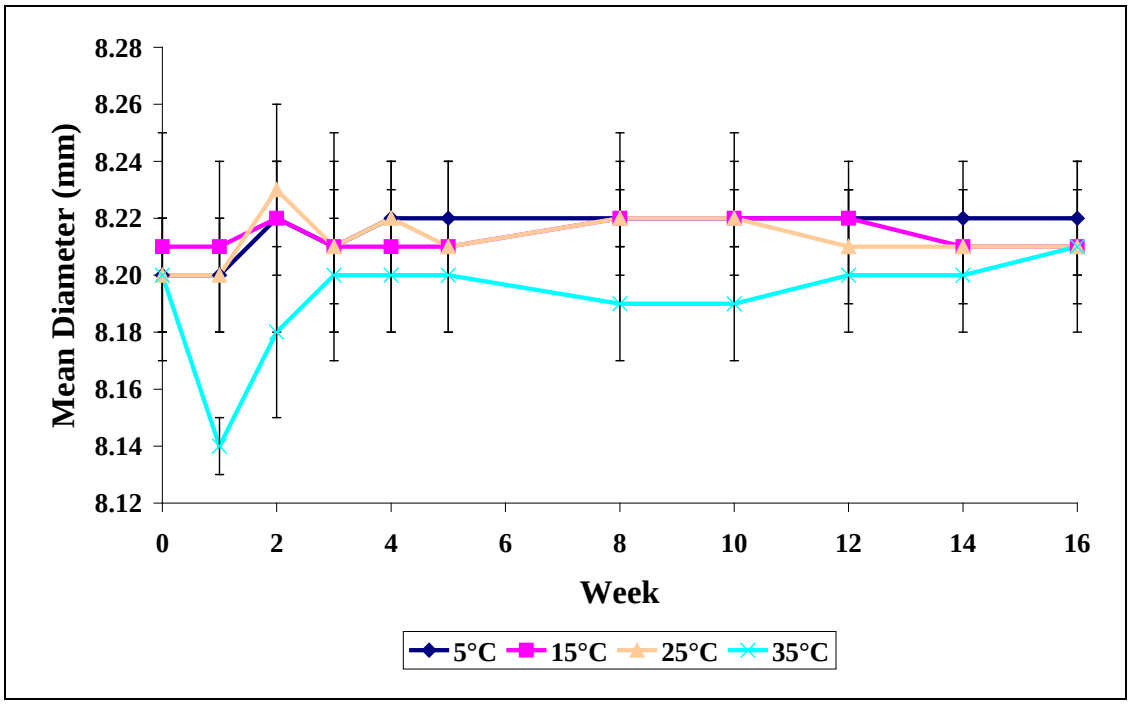
Tablets diameter

At the start of the experiment (week 0) there was no difference ($F(3,36) = 0.416$, $p = 0.743$, one-way ANOVA) between the mean diameter of the tablets in the 4 samples which were then stored at 33% RH at different temperatures (5, 15, 25 and 35°C). There was no significant change in the mean diameter ($p > 0.05$, one-way ANOVA) of 3 out of 4 samples of tablets when stored at 33% RH and at temperatures of 15, 25 and 35°C after 16 weeks when compared to the mean diameter of the separate samples at the start of the experiment (week 0) (Figure 4.8). However, there was significant increase ($p = 0.042$, one-way ANOVA) after 16 weeks in the mean diameter at 5°C when stored at 33% RH when compared with week 0. The difference between the mean diameter of the 4 separate samples of tablets stored at 33% RH and all temperatures (5, 15, 25 and 35°C) for each week, were found to be significant ($p < 0.05$, one-way ANOVA) for weeks 1, 2, 8 and 10 but not significant ($p > 0.05$, one-way ANOVA) for the other weeks (see Table 1.3, Appendix 2).

From the Figure 4.8 (A, B), it could be observed there was a slight change in the mean diameter of tablets at 15°C when stored at 33% RH over 16 weeks. There was a trend to a slight increase in the mean diameter for tablets at 5 and 25°C after week 2. However, while for the tablets at 5°C the mean diameter stabilised after week 4, for the tablets at 25°C there was a slight decrease at the end of week 16 when stored at 33% RH. For the tablets at 35°C there was a decrease in the mean diameter after week 1 and then at week 3 the mean diameter was again similar to week 0 where it appeared to stabilise (Figure 4.8, A).

At the start of the experiment (week 0) there was no difference ($F(3,36) = 0.402$, $p = 0.752$, one-way ANOVA) between the mean diameter of the tablets in the 4 samples which were then stored at 75% RH and different temperatures (5, 15, 25 and 40°C). There was significant increase in the mean diameter ($p < 0.05$, one-way ANOVA) of the 4 separate samples of tablets when stored at 75% RH at all temperatures (5, 15, 25 and 40°C) after 16 weeks when compared with the mean diameter of the separate samples at

A



B

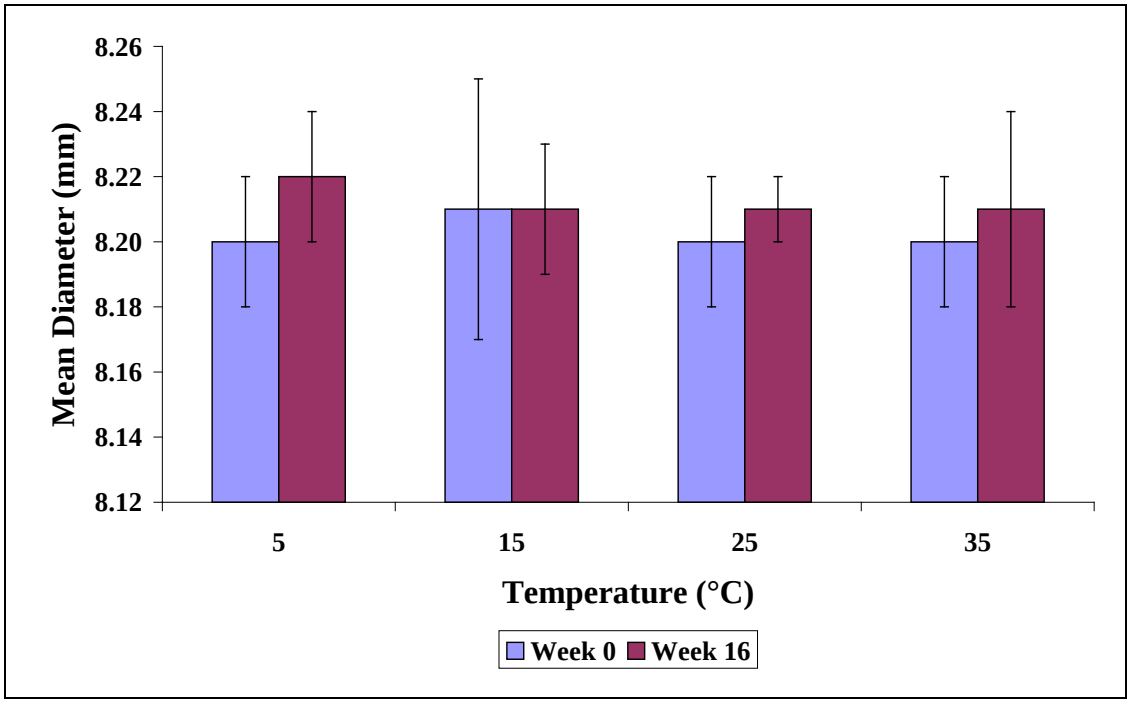
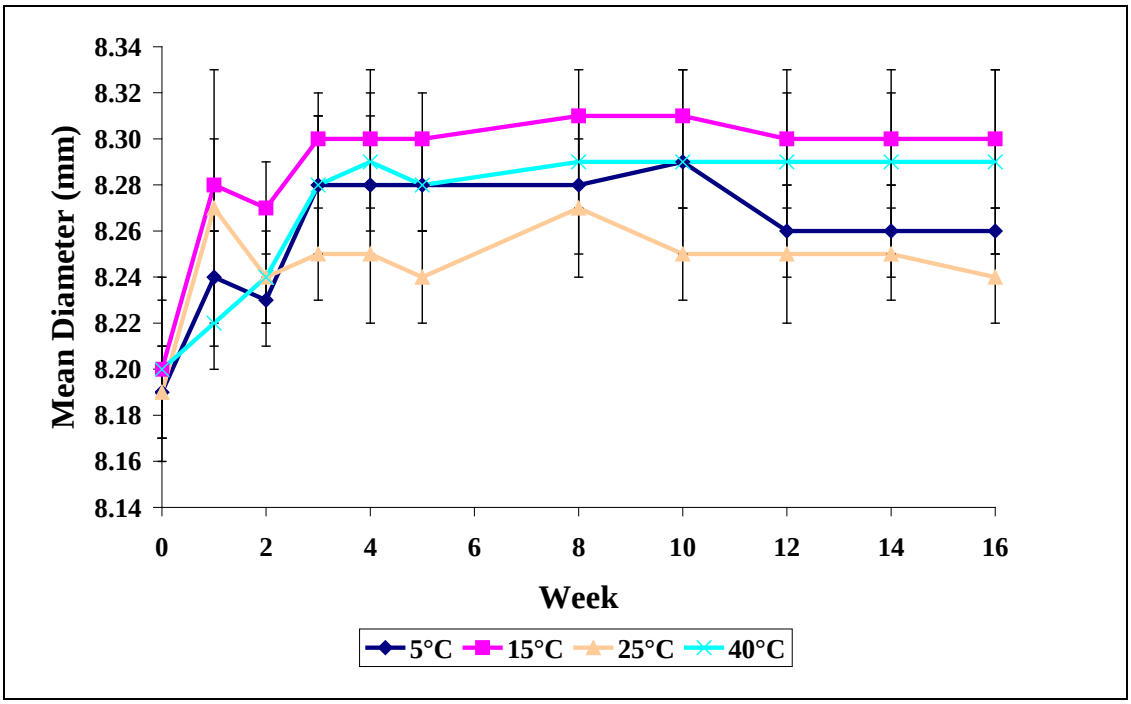


Figure 4.8 A: Mean diameter (mm) of 'ecstasy' tablets exposed to 5, 15, 25 and 35°C and 33% RH between 0 and 16 weeks (Errors bars overlap). B: Comparison of mean diameter at weeks 0 and 16. (Error bars in A and B represent the SD of each mean).

A



B

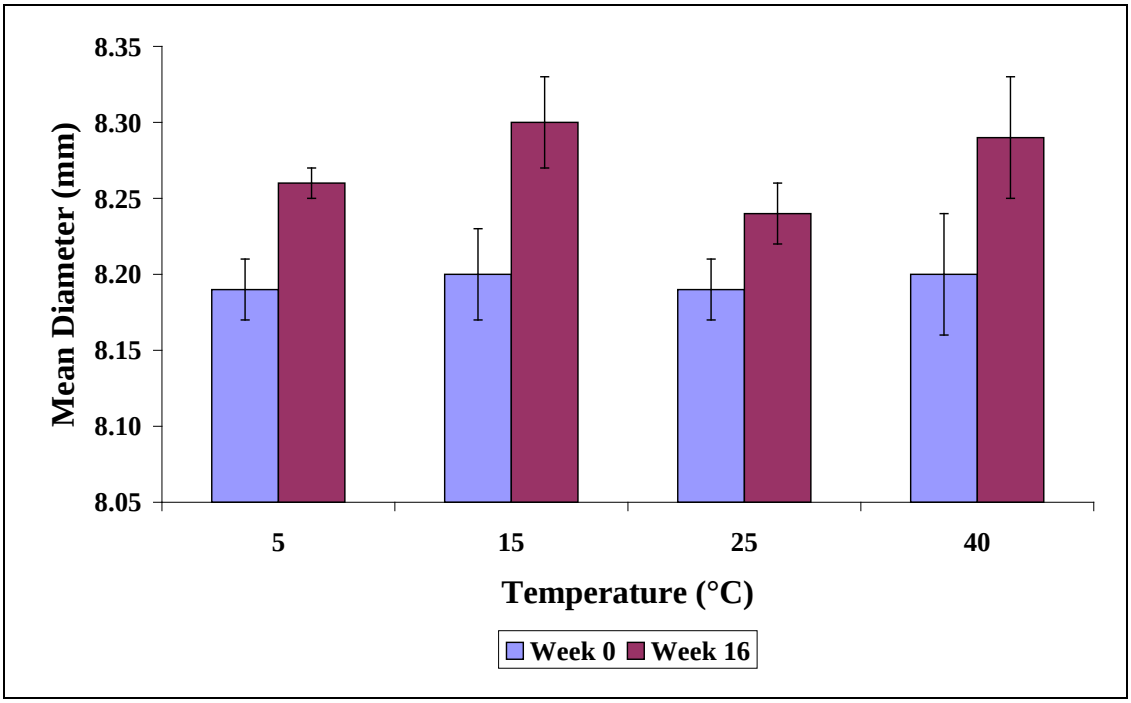


Figure 4.9 A: Mean diameter (mm) of 'ecstasy' tablets exposed to 5, 15, 25 and 40°C and 75% RH between 0 and 16 weeks (Errors bars overlap). B: Comparison of mean diameter at weeks 0 and 16. (Error bars in A and B represent the SD of each mean).

the start of the experiment (week 0) (Figure 4.9, see Table 1.3, Appendix 2). The increase in mean diameter was most prominent in the samples of tablets stored at 75% RH and separate temperatures of 15 and 40°C (increase of 1.22 mm at 15°C and 1.10 mm at 40°C, Figure 4.9, B). The difference between the mean diameter of the 4 separate samples of tablets stored at 75% RH and all temperatures (5, 15, 25 and 40 °C) for each week, between 1 to 16 weeks, was found to be significant ($p < 0.05$, one-way ANOVA) (see Table 1.3, Appendix 2).

When looking at Figure 4.9 (A) it could be concluded that there was a change in the mean diameter of tablets at all temperatures (5, 15, 25 and 40°C) when stored at 75% RH over 16 weeks. The increase in the mean diameter was sustained over several weeks at the beginning of the study, up to week 3 for many temperatures and continuing in tablets exposed to 25°C for 8 weeks. For the tablets stored at 15 and 40°C at 75% RH after increase in the mean diameter at weeks 3 and 4 respectively, stabilised to week 16. The tablets at 5°C, after the initial increase in the mean diameter stabilised till week 10 when it then decreased and stabilised till week 16. The mean diameter of tablets at 25°C after the increase at week 8 started to decrease till week 16 (Figure 4.9, A). The mean diameter of the tablets at 16 weeks at all temperatures at 75% RH had increased when compared with the mean diameter at the start of the exposure (Figure 4.9, A).

At week 16 there was significant increase in the mean diameter of the separate samples of tablets stored at 75% RH and temperatures of 5, 15 and 25°C ($p < 0.05$, one-way ANOVA) when compared with the mean diameter of tablets in the separate samples stored at 33% RH and similar temperatures (Figure 4.10). There was also significant increase in the mean diameter of the tablets stored at 40°C at 75% RH when compared with the mean diameter of the tablets stored at 35°C at 33% RH ($p < 0.05$, one-way ANOVA) (Figure 4.10). The most increase in the mean diameter of tablets stored at 75% RH when compared with the tablets stored at 33% RH occurred with the tablets at 15 and 40°C (an increase of 1.10 mm at 15°C and 0.97 mm at 40°C) (Figure 4.10, see Table 1.3, Appendix 2). If only the RH is considered the mean diameter of the total tablets (n = 40) in the 4 samples stored at RH of 33% had a relatively marked increase in the diameter

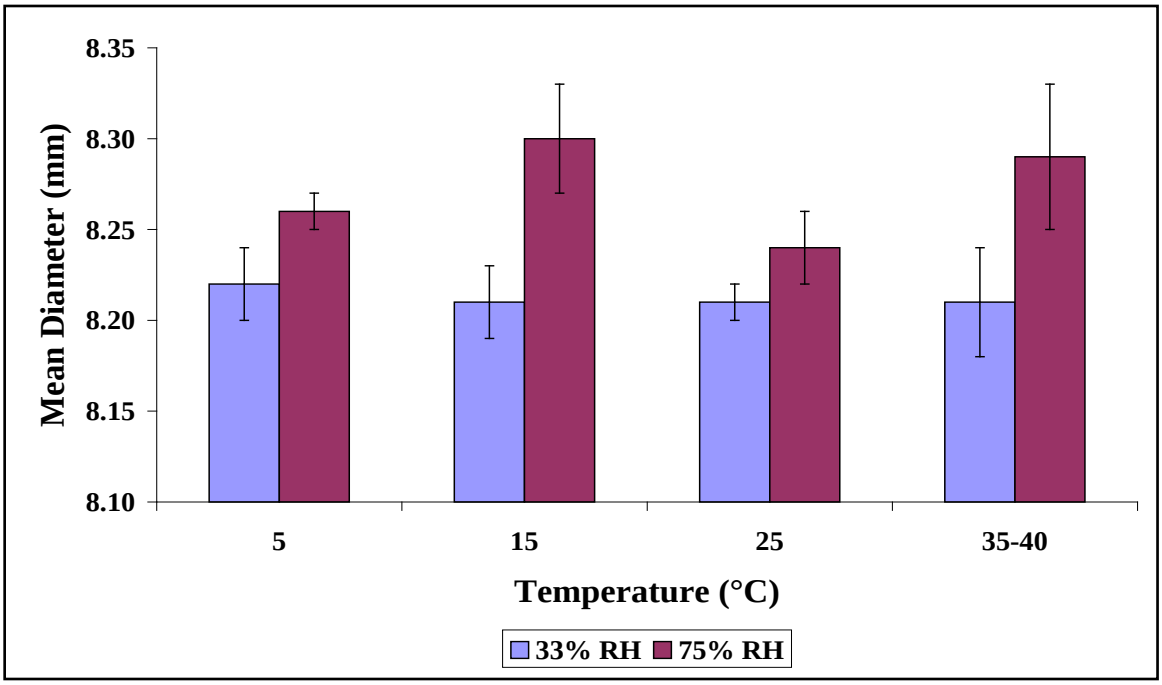


Figure 4.10 Mean diameter (mm) of 'ecstasy' tablets exposed to 5, 15, 25 and 35°C and 33% RH and at 5, 15, 25 and 40°C and 75% RH at week 16 (Error bars represent the standard deviation of each mean).

after week 2 and remained rather constant thereafter. Furthermore, if week 1 is excluded, no significant difference existed between the mean diameter of all the tablets in the 4 samples stored at RH of 33% from week 0 to week 2 through to week 16. (F (9, 390) = 0.720, p = 0.690, one-way ANOVA) (Figure 4.11).

Whereas the mean diameter of all the tablets (n = 40) in the 4 samples that were stored at 75% RH had significantly increased from week 0 to week 3 and remained more or less constant to week 16 (F (10, 429) = 27.199, $p < 0.001$, one-way ANOVA). There was also a significant increase in the mean diameter of the total tablets (n = 40) that were stored at 75% RH and all temperatures (5, 15, 25 and 40°C) for weeks from 3 to 16 when compared to week 2 ($p > 0.05$, one-way ANOVA) (Figure 4.11).

A Tukey post-hoc test indicated that the mean diameter of all the tablets in the 4 samples stored at RH of 75% after 1 week were significant wider (8.25 mm SD 0.04, $p < 0.001$, one-way ANOVA) than at week 0 (8.19 mm SD 0.03) and that there was further increase

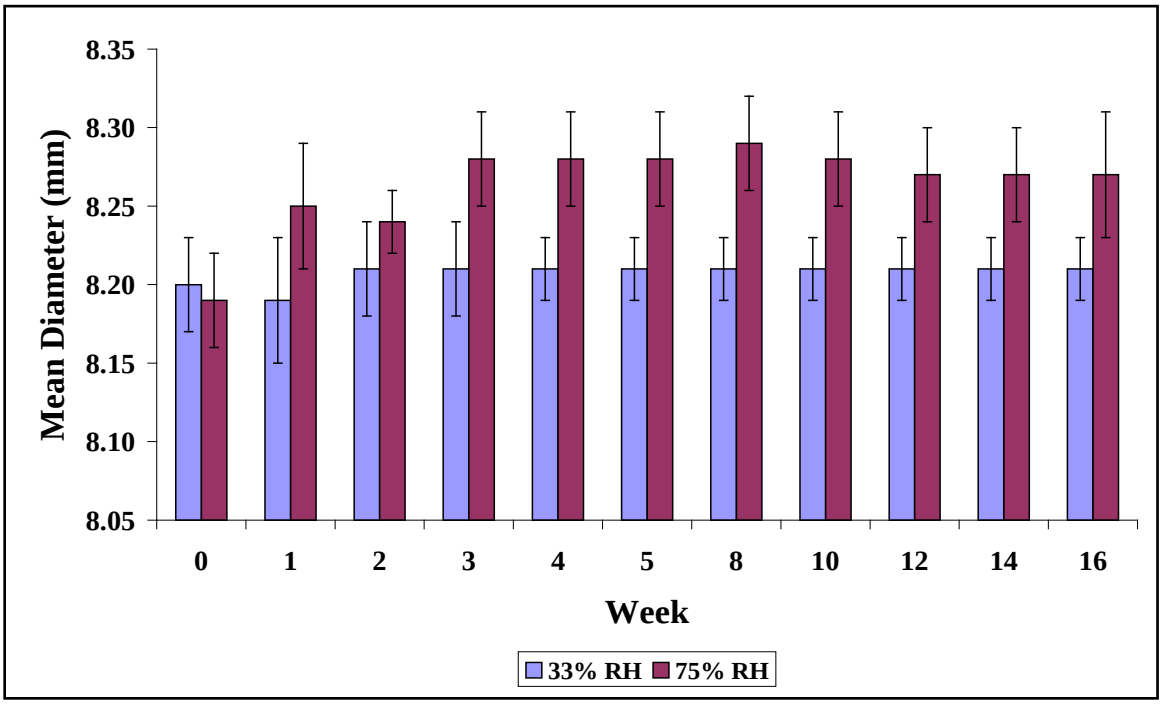


Figure 4.11 Mean diameter (mm) of total 'ecstasy' tablets (n = 40, 10 tablets in each of the 4 samples) exposed to 33% RH and mean diameter of total 'ecstasy' tablets (n = 40) exposed at 75% RH between 0 and 16 weeks (Error bars represent the SD of each mean).

in the width to week 16 (8.27mm SD 0.04, $p < 0.001$, one-way ANOVA, increase of 0.98%). The tolerance limit for the mean diameter of all the tablets in the 4 samples stored at RH of 75% using the tolerance factor k for a two-sided test, (95% CI and 99% coverage) changed from 8.19 mm (SD 0.10) at week 0 to 8.27 mm (SD 0.11) at week 16 (Figure 4.11, see Table 1.4, Appendix 2).

Considering only the RHs there no significant difference between the mean diameter of all tablets (n = 40) stored at 75% RH when compared with all tablets at 33% RH at week 0 ($p > 0.05$, one-way ANOVA). However there was significant increase ($p < 0.05$, one-way ANOVA) for each week (between week 1 to 16) in the mean diameter of all the tablets in the 4 samples (n = 40) stored at 75% RH when compared with the mean diameter of all tablets (n = 40) stored at 33% RH (Figure 4.11, see Table 1.4, Appendix 2). There was an increase of 0.12% in the mean diameter of the total tablets stored at

33% RH after 16 weeks, and an increase of 0.98% in the mean diameter of the total tablets stored at RH of 75% (Figure 4.11).

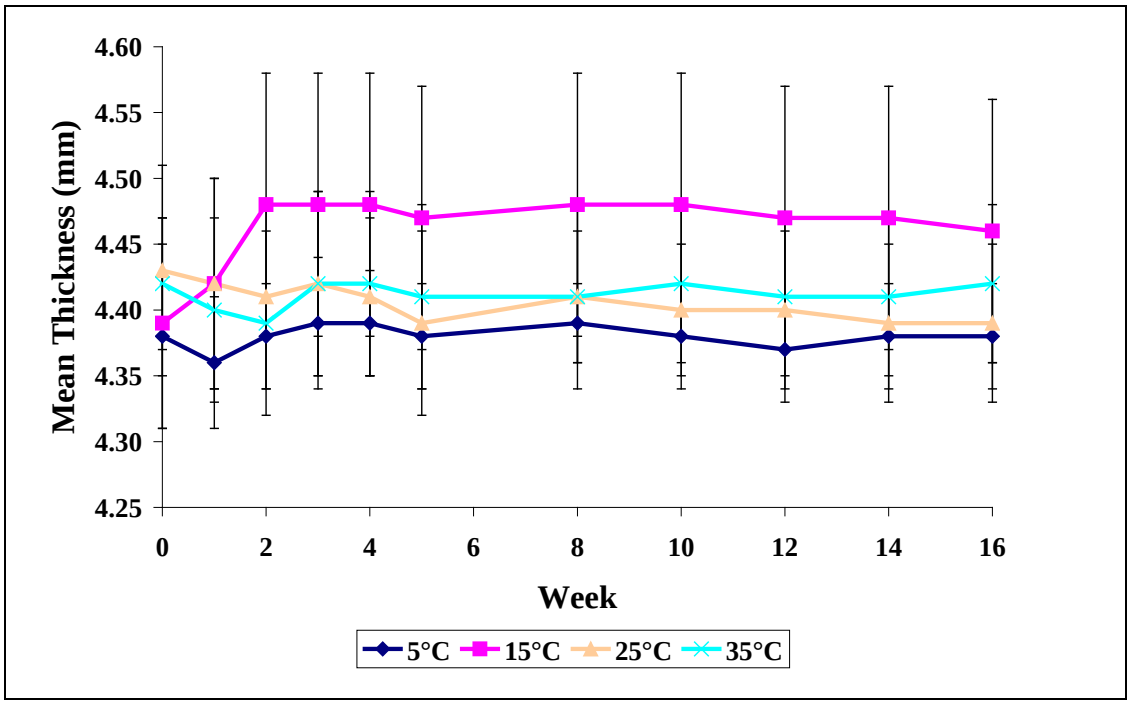
Tablets thickness

At the start of the experiment (week 0) there was no significant difference ($F(3,36) = 0.847$, $p = 0.477$, one-way ANOVA) between the mean thickness of the tablets in the 4 samples which were stored at 33% RH and separate temperatures (5, 15, 25 and 35°C). However, significant difference were observed between the mean thickness of the 4 separate samples of tablets stored at 33% RH and all temperatures (5, 15, 25 and 35°C) ($p < 0.05$, one-way ANOVA) for each week with the exception of weeks 1 and 3 were there was no significant difference ($p > 0.05$, one-way ANOVA) (see Table 1.5, Appendix 2).

From the Figure 4.12 (A), it could be concluded there was slight change in the mean thickness of tablets at 5 and 35°C when stored at 33% RH over 16 weeks. The mean thickness of the samples of tablets at 25°C had slightly decreased, while those at 15°C had increased when stored at 33% RH during the 16 weeks. It was also noted that the increase in the mean thickness for the tablets at 15°C had occurred in the first two weeks (Figure 4.12, A). There was no significant change in the mean thickness ($p > 0.05$, one-way ANOVA) of the 4 separate samples of tablets when stored at 33% RH and all temperatures (5, 15, 25 and 35°C) at the end of the study after 16 weeks when compared to the mean thickness of the separate samples at the start of the experiment (week 0) (Figure 4.12, B).

At the start of the experiment (week 0) there was no difference ($F(3,36) = 0.467$, $p = 0.707$, one-way ANOVA) between the mean thickness of the tablets in the 4 samples which were then stored at 75% RH and different temperatures (5, 15, 25 and 40°C). There was significant increase in the mean thickness ($p < 0.05$, one-way ANOVA) of the 4 separate samples of tablets when stored at 75% RH and all temperatures (5, 15, 25 and 40°C) after the initial increase in the first 2 weeks and after 16 weeks when compared

A



B

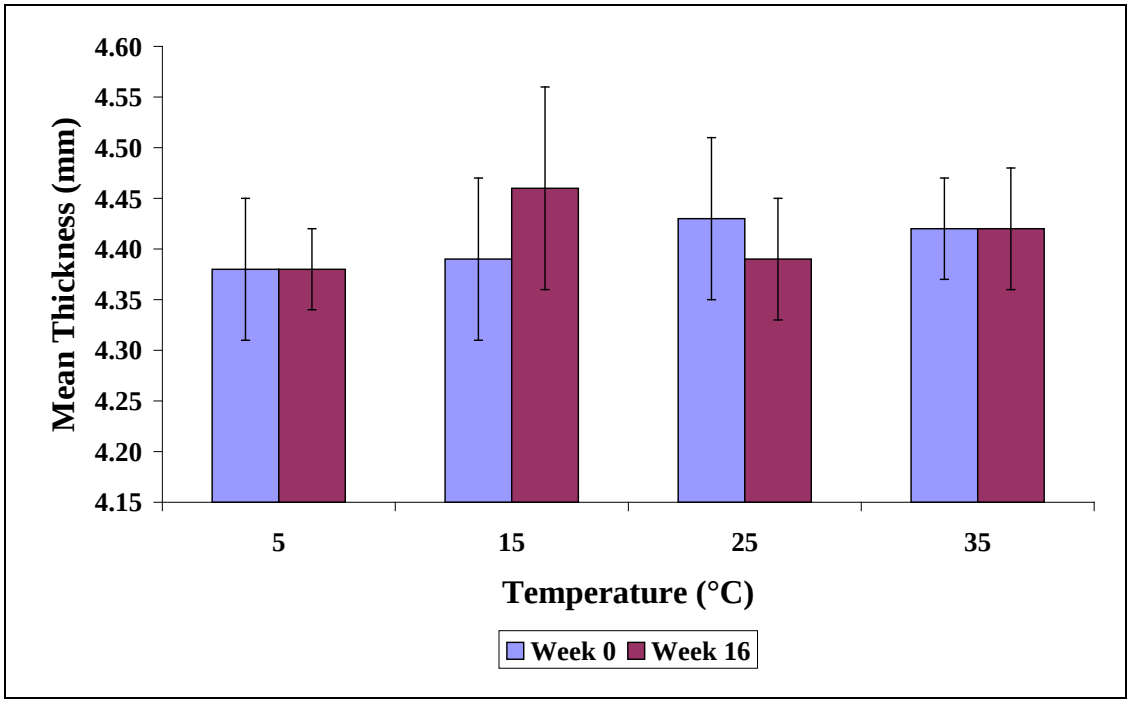
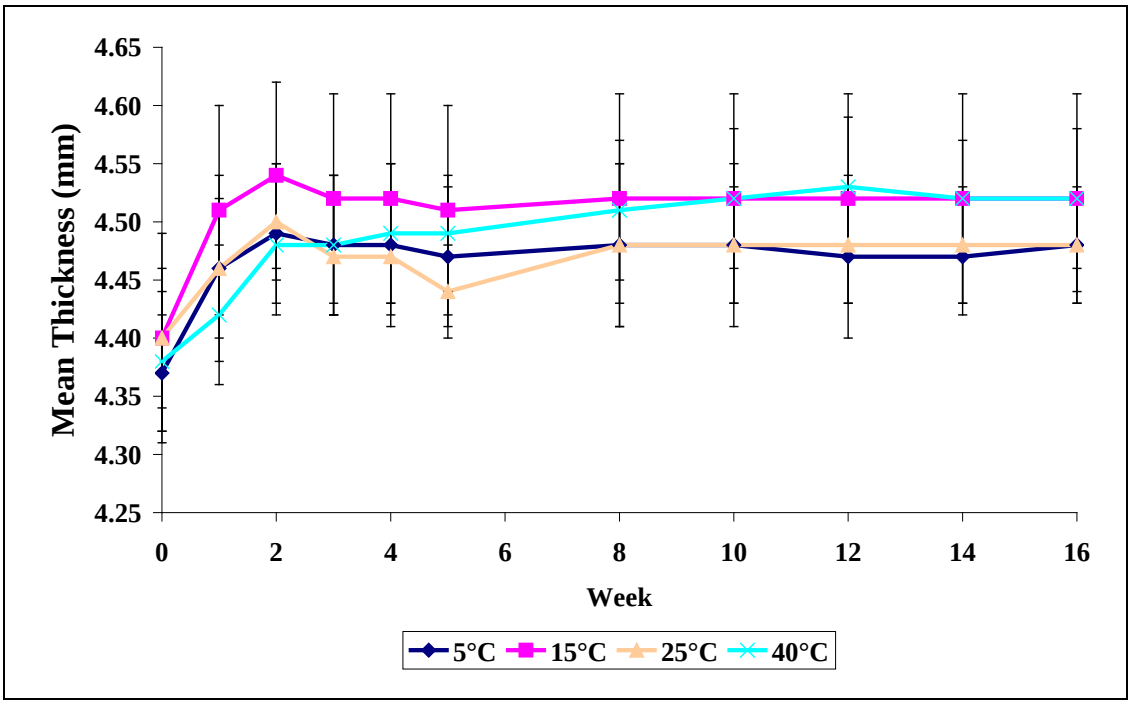


Figure 4.12 A: Mean thickness (mm) of 'ecstasy' tablets exposed to 5, 15, 25 and 35°C and 33% RH between 0 and 16 weeks (Errors bars overlap). B: Comparison of mean thickness at weeks 0 and 16. (Error bars in A and B represent the SD of each mean).

A



B

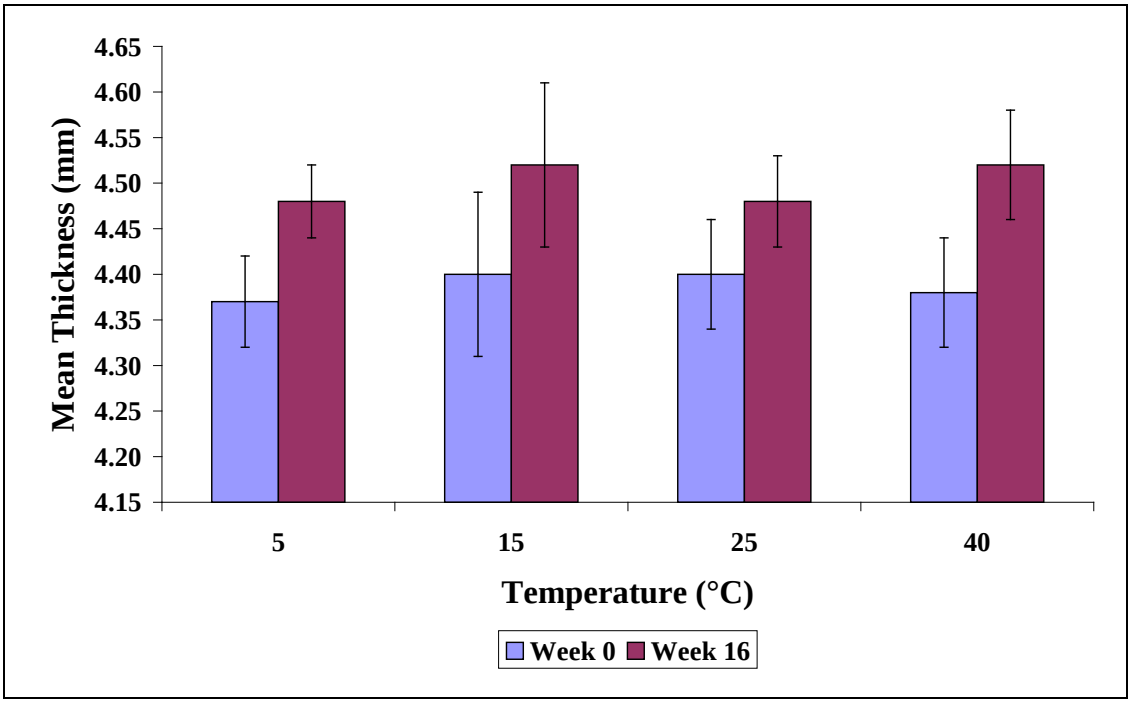


Figure 4.13 A: Mean thickness (mm) of 'ecstasy' tablets exposed to 5, 15, 25 and 40°C and 75% RH between 0 and 16 weeks (Errors bars overlap). B: Comparison of mean thickness at weeks 0 and 16. (Error bars in A and B represent the SD of each mean).

with the mean thickness of the separate samples at the start of the experiment (week 0) (Figure 4.13) (see Table 1.5, Appendix 2). The increase in the mean thickness was most prominent in the samples of tablets stored at 75% RH and separate temperatures of 15 and 40°C (increase of 0.12 mm at 15°C and 0.14 mm at 40°C, Figure 4.13, B) (see Table 1.5, Appendix 2). The difference between the mean thickness of the 4 separate samples of tablets stored at 75% RH and all temperatures (5, 15, 25 and 40 °C) for each week, between 1 to 16 weeks, were not found to be significant ($p > 0.05$, one-way ANOVA) (see Table 1.5, Appendix 2).

Figure 4.13 (A) demonstrates that there was a change in the mean thickness of tablets at all temperatures (5, 15, 25 and 40°C) when stored at 75% RH over 16 weeks, with the increase in the mean thickness occurring within the first two weeks for all temperatures. For the tablets stored at 5, 15 and 25°C at 75% RH after the initial increase in the mean thickness in the first two weeks, there appears to be a very slight decrease in the thickness between 2 to 16 weeks. For the tablets stored at 40°C, the increase in thickness was most marked in the first two weeks and continued to week 12 were it appeared to stabilise. The mean thickness of the tablets at 16 weeks at all temperatures and stored at 75% RH had increased when compared with the mean thickness at the start of the exposure (Figure 4.13, A).

At week 16 there was significant increase in the mean thickness of the separate samples of tablets stored at 75% RH and 5 and 25°C ($p < 0.05$, one-way ANOVA) when compared with the mean thickness of the tablets in the separate samples stored at 33% RH and similar temperatures (Figure 4.14). There was also significant increase in the mean thickness of the tablets stored at 40°C at 75% RH when compared with the mean thickness of the tablets stored at 35°C at 33% RH ($p = 0.003$, one-way ANOVA) (Figure 4.14). However, there was no significant difference between the mean thickness of tablets stored at 15°C at 33 and 75% RH ($p = 0.478$, one-way ANOVA). The greatest increase in the mean thickness of the tablets stored at 75% RH when compared with tablets stored at 33% RH occurred at 5 and 40°C (an increase of 0.10 mm for both temperatures) (Figure 4.14, see Table 1.5, Appendix 2).

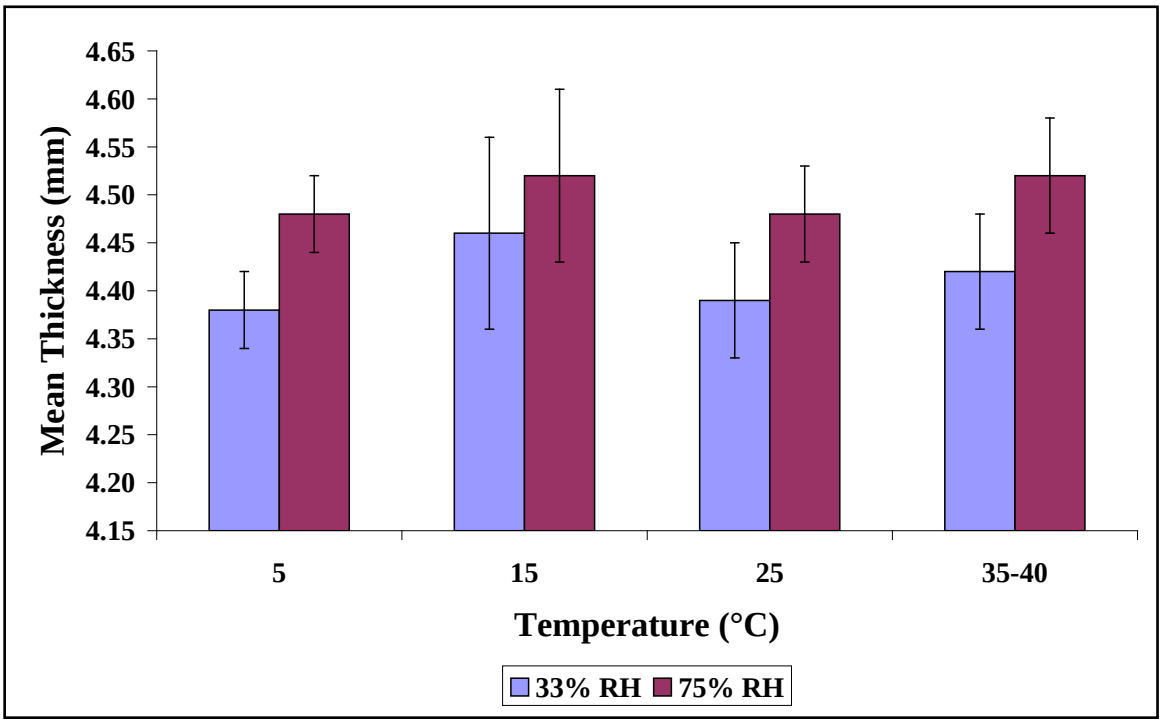


Figure 4.14 Mean thickness (mm) of 'ecstasy' tablets exposed to 5, 15, 25 and 35°C and 33% RH and at 5, 15, 25 and 40°C and 75% RH at week 16 (Error bars represent the SD of each mean).

There was no significant difference between the mean thickness of the total tablets (n = 40) stored at RH of 33% during the study period ($F(10, 429) = 0.473$, $p = 0.908$, one-way ANOVA) (see Table 1.6, Appendix 2). However, there was significant increase in the mean thickness of the total tablets (n = 40) that were stored at 75% RH for weeks from 1 to 16 when compared to week 0 (F (10, 429) = 9.484, p <0.001, one-way ANOVA). Moreover, there was no significant difference between the mean thickness of the total tablets (n = 40) that were stored at 75% RH and all temperatures (5, 15, 25 and 40°C) between 1 to 16 weeks ($p > 0.05$, one-way ANOVA).

A Tukey post-hoc test revealed that after 1 week the mean thickness of tablets stored at 75% RH had significantly increased (4.46 mm SD 0.08, $p < 0.001$, one-way ANOVA) and remained so even after 16 weeks (4.50 mm SD 0.07, $p < 0.001$, one-way ANOVA) when compared to the tablets thickness at week 0 (4.39 mm SD 0.07) (see Table 1.6, Appendix 2) an increase of 2.51%. The tolerance limit using the tolerance factor k for a

two-sided test (95% CI and 99% coverage) changed from 4.39 mm (SD 0.21) for week 0 to 4.50 mm (SD 0.22) at week 10 and to 4.50 mm (SD 0.21) at week 16 for the tablets stored at 75% RH (Figure 4.15).

There was significant increase ($p < 0.05$, one-way ANOVA) for each week (between week 1 to 16) in the mean thickness of all the tablets in the 4 samples (n = 40) stored at 75% RH when compared with the mean thickness of all tablets (n = 40) stored at 33% RH (Figure 4.15, see Table 1.6, Appendix 2), when RH was considered as the sole parameter.

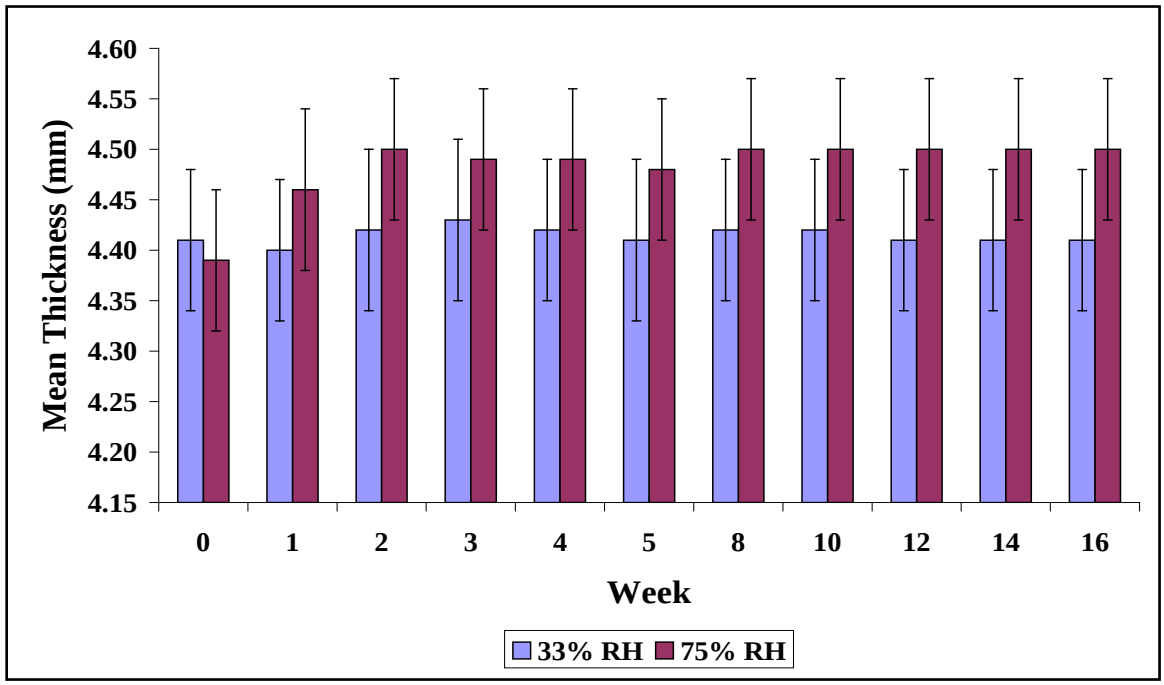


Figure 4.15 Mean thickness (mm) of total 'ecstasy' tablets (n = 40, 10 tablets in each of the 4 samples) exposed to 33% RH and mean thickness of total 'ecstasy' tablets (n = 40) exposed at 75% RH between 0 and 16 weeks (Error bars represent the SD of each mean).

Tablets volume

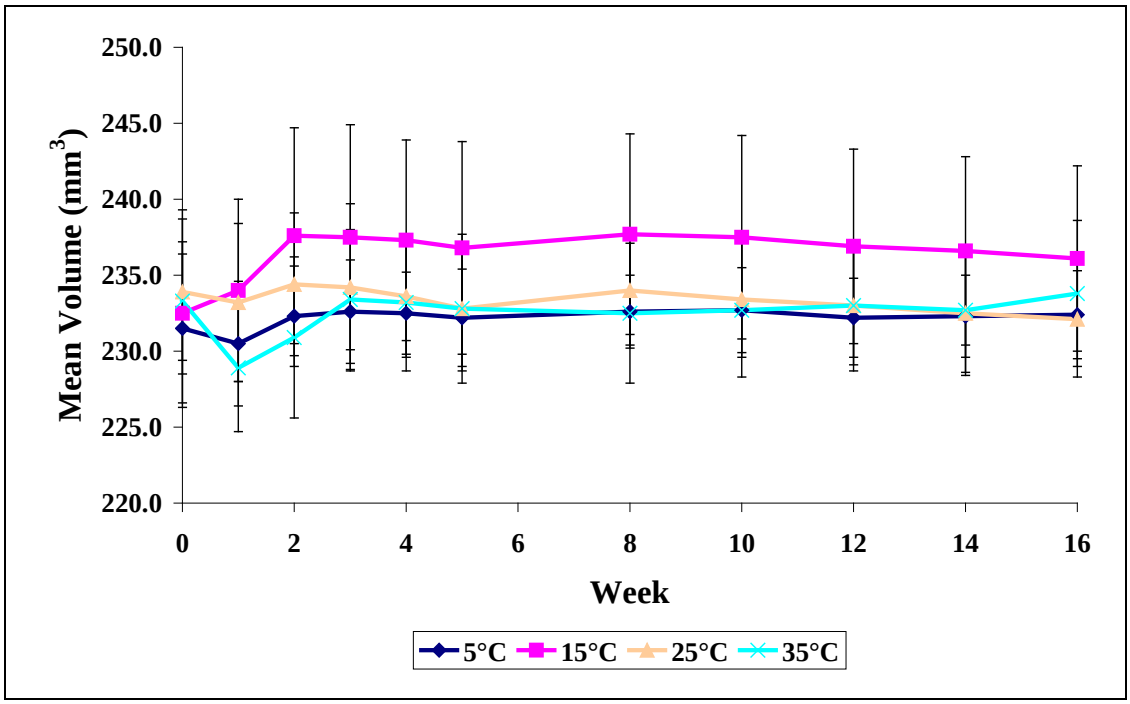
At the start of the experiment (week 0) there was no difference ($F(3,36) = 0.389$, $p = 0.762$, one-way ANOVA) between the mean volume of the tablets in the 4 samples stored

at 33% RH and separate temperatures (5, 15, 25 and 35°C). There was no significant change in the mean volume ($p > 0.05$, one-way ANOVA) of the 4 separate samples of tablets when stored at 33% RH and at temperatures of 5, 15, 25 and 35°C after 16 weeks when compared to the mean volume of the separate samples at the start of the experiment (week 0) (Figure 4.16). The difference between the mean volume of the 4 separate samples of tablets stored at 33% RH and all temperatures (5, 15, 25 and 35°C) for each week, was not significant ($p > 0.05$, one-way ANOVA and Welch test) (see Table 1.7, Appendix 2).

From the Figure 4.16 (A), it could be concluded that at week 1 there was a slight decrease in the mean volume of tablets at 5°C and a decrease in the mean volume of tablets stored at 33% RH and 35°C. However, at weeks 2 and 3 respectively the mean volume of tablets at 5 and 35°C was again similar to week 0 where it stabilised to week 16. There was an initial increase in the mean volume of the tablets at 15°C and 33% RH after week 2 and then stabilising to week 16. For the tablets at 25°C stored at 33% RH there was little change in the mean volume over 16 weeks (Figure 4.16, A).

At the start of the experiment (week 0) there was no difference ($F(3,36) = 0.287$, $p = 0.835$, one-way ANOVA) between the mean volume of the tablets in the 4 samples which were then stored at 75% RH and separate temperatures (5, 15, 25 and 40°C). There was a significant increase in the mean volume ($p > 0.05$, one-way ANOVA) of the 4 separate samples of tablets when stored at 75% RH and all temperatures (5, 15, 25 and 40°C) after 16 weeks when compared with the mean volume of the separate samples at the start of the experiment (week 0) (Figure 4.17, see Table 1.7, Appendix 2). The increase in mean volume was most prominent in the samples of tablets stored at 75% RH and at 40 and 15°C (increase of 13.1 mm^3 at 40°C and 11.9 mm^3 at 15°C, Figure 4.17, B). The difference between the mean volume of the 4 separate samples of tablets stored at 75% RH and all temperatures (5, 15, 25 and 40 °C) for each week, between 1 to 16 weeks, was found to be significant ($p < 0.05$, one-way ANOVA) for weeks 1, 5, 12 to 16 and not significant for the other weeks (see Table 1.7, Appendix 2).

A



B

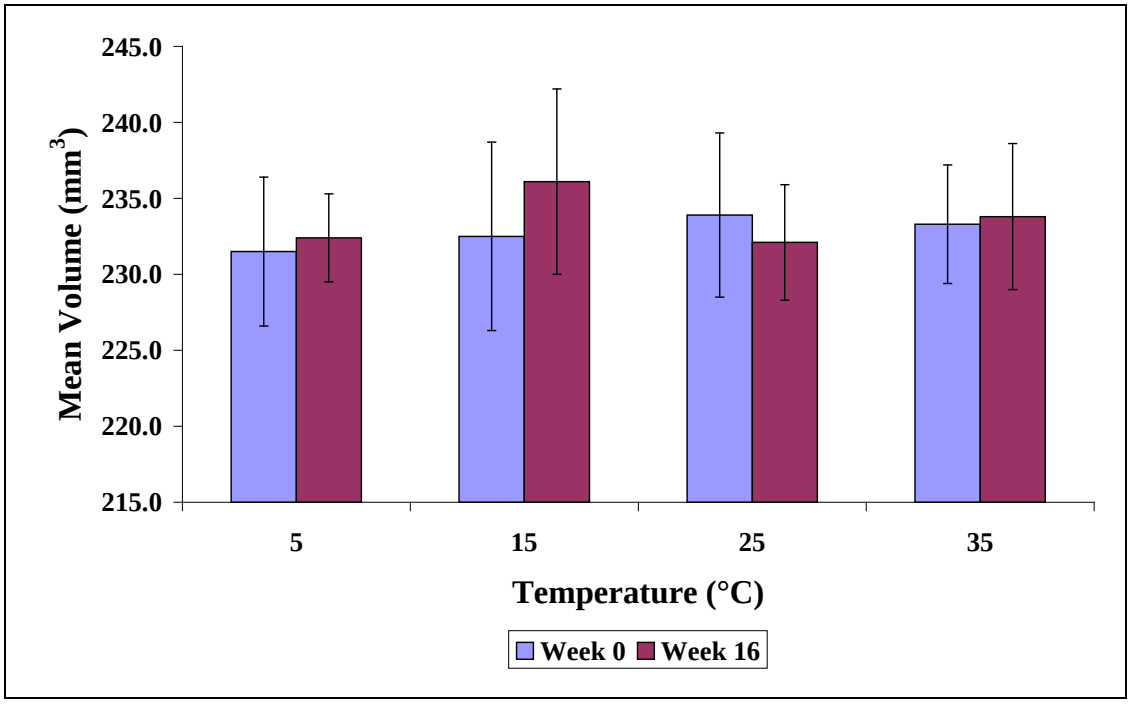
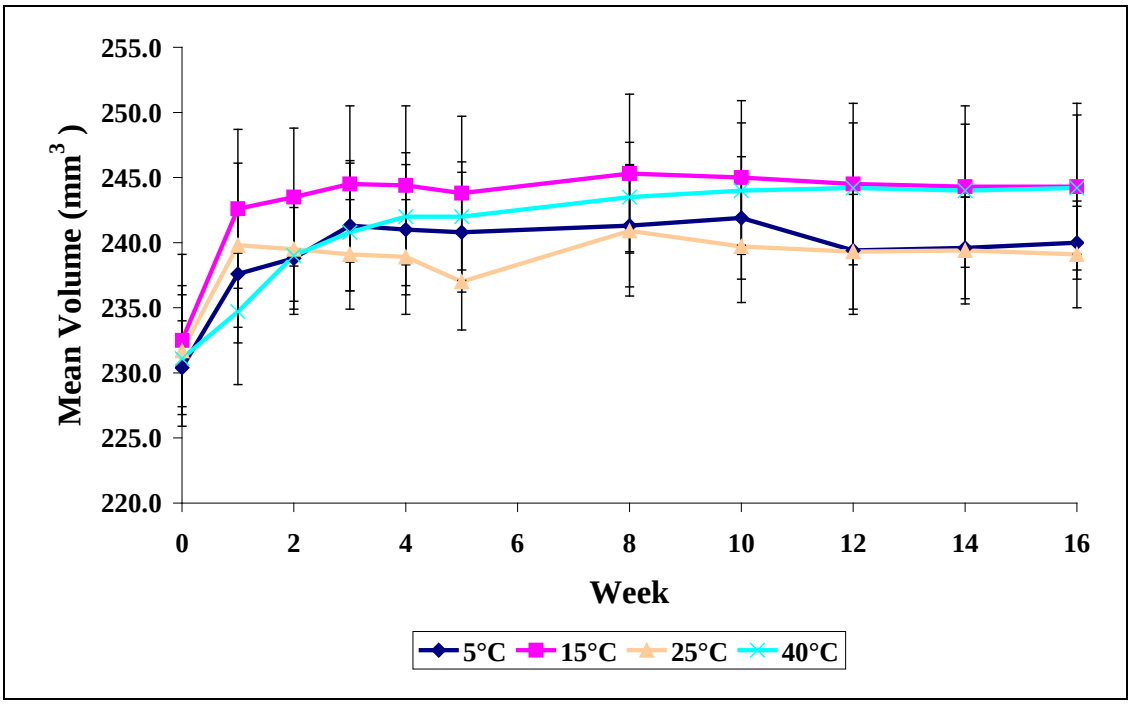


Figure 4.16 A: Mean volume (mm^3) of 'ecstasy' tablets exposed to 5, 15, 25 and 35°C and 33% RH between 0 and 16 weeks (Errors bars overlap). B: Comparison of mean volume at weeks 0 and 16. (Error bars in A and B represent the SD of each mean).

A



B

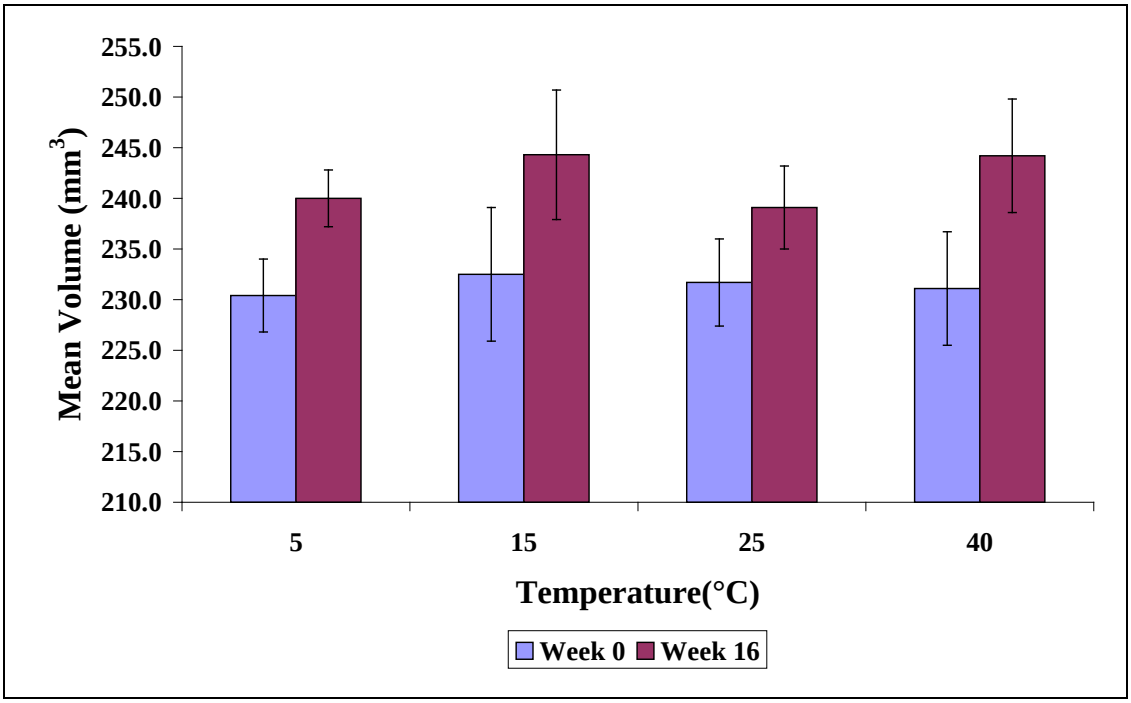


Figure 4.17 A: Mean volume (mm^3) of 'ecstasy' tablets exposed to 5, 15, 25 and 40°C and 75% RH between 0 and 16 weeks (Errors bars overlap). B: Comparison of mean volume at weeks 0 and 16. (Error bars in A and B represent the SD of each mean).

When looking at Figure 4.17 (A) it could be concluded that there was a change in the mean volume at all temperatures (5, 15, 25 and 40°C) when stored at 75% RH over 16 weeks. The increase in the mean volume for the tablets at 5, 15 and 25°C occurred at week 1, and for tablets at 40°C occurred at week 3 and went on until week 12 (Figure 4.17, A). For the tablets stored 15°C at 75% RH after the initial increase in the mean volume at week 1 stabilised to week 16. The mean volume of the tablets at 16 weeks at all temperatures at 75% RH had significantly increased ($p \leq 0.004$, one way ANOVA) when compared to the mean volume at the start of the exposure (Figure 4.17, A).

At week 16 there was significant increase in the mean volume of the separate samples of tablets stored at 75% RH and 5, 15 and 25°C ($p < 0.05$, one-way ANOVA) when compared with the mean volume of tablets in the separate samples stored at 33% RH and similar temperatures (Figure 4.18). There was also a significant increase in the mean

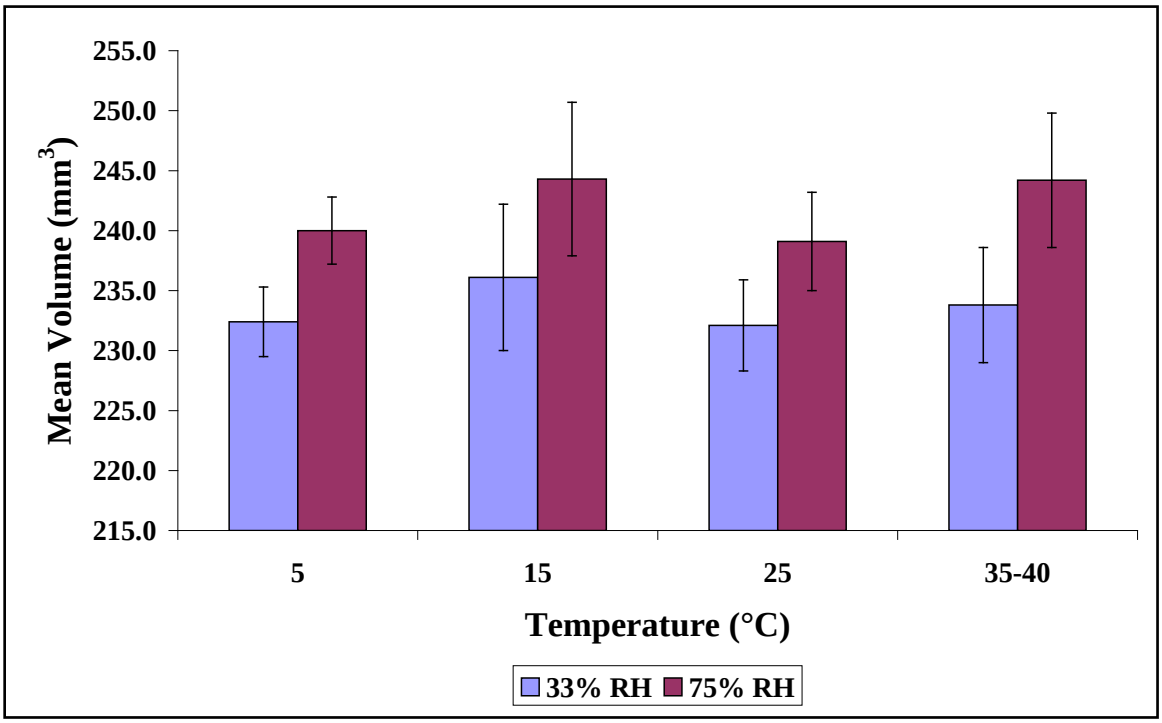


Figure 4.18 Mean volume (mm^3) of 'ecstasy' tablets exposed to 5, 15, 25 and 35°C and 33% RH and at 5, 15, 25 and 40°C and 75% RH at week 16 (Error bars represent the standard deviation of each mean).

volume of the tablets stored at 40°C at 75% RH when compared with the mean volume of the tablets stored at 35°C at 33% RH ($p < 0.05$, one-way ANOVA). The greatest increase in the mean volume of tablets stored at 75% RH when compared with tablets stored at 33% RH occurred with the tablets at 40 and 15°C (an increase of 10.4 mm^3 at 40°C and 8.3 mm^3 at 15°C, Figure 4.18, see Table 1.7, Appendix 2).

If only the RH is considered the mean volume of the total tablets (n = 40) stored at 33% RH had a relatively marked increase in the volume after week 3 and remained rather constant thereafter. There was no difference in the mean volume of all tablets (n = 40) stored at 33% RH when compared for each week, from week 0 to 16 (F (10, 428) = 0.953, p = 0.485, one-way ANOVA) (Figure 4.19).

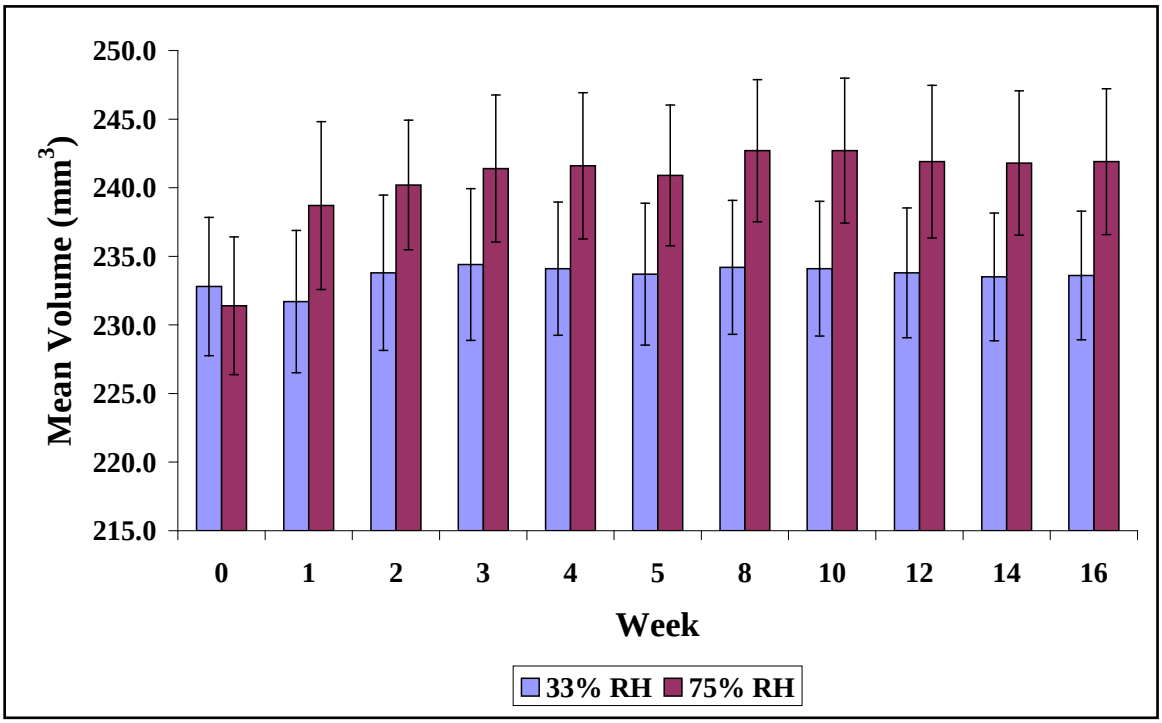


Figure 4.19 Mean volume (mm^3) of total 'ecstasy' tablets (n = 40, 10 tablets in each of the 4 samples) exposed to 33% RH and mean volume of total 'ecstasy' tablets (n = 40) exposed at 75% RH between 0 and 16 weeks (Error bars represent the SD of each mean).

The mean volume of all the tablets (n = 40) that were stored at 75% RH had significantly increased from week 0 to week 1 and remained more or less constant to week 16 (F (10,

429) = 14.67, $p < 0.001$, one-way ANOVA) when RH was considered as the sole parameter. A Tukey post-hoc test indicated that the mean volume of all the tablets (n = 40) stored at RH of 75% after 1 week had significantly swelled (238.7 mm^3 SD 6.12, $p < 0.001$, one-way ANOVA) when compared with week 0 (231.4 mm^3 SD 0.03) and that there was further significant increase in the volume to week 16 (241.9 mm SD 5.32, $p < 0.001$, one-way ANOVA). (Figure 4.19, see Table 1.8, Appendix 2).

There was significant increase ($p < 0.05$, one-way ANOVA) for each week (between week 1 to 16) in the mean volume of all the tablets in the 4 samples (n = 40) stored at 75% RH when compared with the mean volume of all tablets (n = 40) stored at 33% RH (Figure 4.19, see Table 1.8, Appendix 2) if only the RH is considered. Moreover, there was an increase of 0.34% in the mean volume of the total tablets stored at 33% RH after 16 weeks, and an increase of 4.54% in the mean volume of the total tablets stored at RH of 75% (Figure 4.19).

Tablets hardness

There was no significant change ($p > 0.05$, one-way ANOVA) in the hardness of tablets stored at 33% RH and temperatures 5, 15 and 35°C respectively with the exception of tablets stored at 25°C ($p = 0.04$, one-way ANOVA) after 16 weeks when compared to week 0 (see Table 1.9, Appendix 2). Also, there was significant decrease ($p < 0.001$, one-way ANOVA) in the hardness of tablets that was stored at 75% RH and temperatures of 5, 15, 25 and 40°C respectively after 16 weeks when compared with week 0 (mean of 47 N at week 0 and means ranging between 37.46 N to < 20 N after 16 weeks) (see Table 1.9, Appendix 2).

Tablets friability

Although the friability tests for the samples of tablets at week 16 could not be calculated because there was not enough tablets in each sample to carry out the test, it was noted

that the tablets stored at 75% RH and temperatures of 5, 15, 25 and 40°C were more friable than the tablets stored at 33% RH and all temperatures (Figure 4.20).

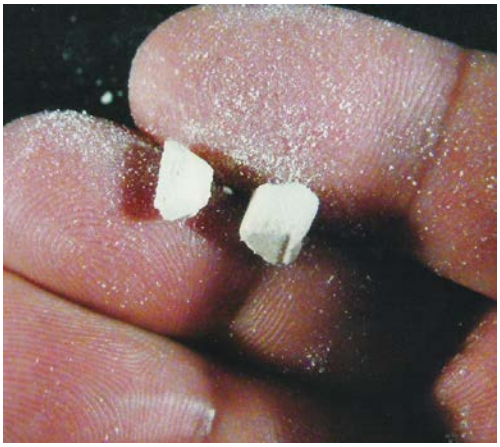


Figure 4.20 Friability of one of the tablets at 40°C and at 75% RH after 16 weeks.

Stability testing: key findings

Mass

- Significant decrease in the mean mass of tablets stored at 33% RH and 35°C;
- Regardless of temperature the tablets stored at 75% RH gained significantly more mass on week by week bases compared to those stored at 33% RH;

Diameter

- Significant increase in the mean diameter of tablets stored at 33% RH and 5°C over 16 weeks;
- Significant increase in the mean diameter of tablets in all samples stored at 75% RH between week 0 an16 regardless of temperature;
- Mean diameter increased most in samples of tablets stored at 75% RH and 15 and 40°C;
- Mean diameter increased more significantly in samples of tablets stored at 75% RH and 15 and 40°C when compared to 33% RH;

Thickness

- Significant increase in the mean thickness of tablets in all samples stored at 75% RH between week 0 and 16 regardless of temperature;
- Mean thickness increased most in samples of tablets stored at 75% RH and 15 and 40°C;
- Significant increase in the mean thickness of samples of tablets stored at 75% RH at 5, 25 and 40°C when compared to 33% RH, not at 15°C;
- Mean thickness increased more significantly in samples of tablets stored at 75% RH and 5 and 40°C when compared to 33% RH;

Volumes

- Significant increase in the mean volume of tablets in all samples stored at 75% RH between week 0 and 16 regardless of temperature;
- Mean volume increased most in samples of tablets stored at 75% RH and 15 and 40°C;
- Regardless of temperature the tablets stored at 75% RH gained significantly more volume on week 16 when compared to those stored at 33% RH;
- Mean volume increased more significantly in samples of tablets stored at 75% RH and 40°C when compared to 33% RH and 35°C;

Hardness

- Significant decrease in the mean hardness of tablets in all samples stored at 75% RH at week 16 when compared to week 0 regardless of temperature;
- Mean hardness decreased most in samples of tablets stored at 75% RH and 40°C.

4.4.4 Comparison between the 'accelerated storage' study and the 'stability' study

The changes occurring in tablets from batches 5, 8 and 11 after 90 and 180 days were compared with changes occurring after 13 weeks (91 days) and 16 weeks (112 days) in

tablets stored at 33 and 75% RH at 35 and 40°C. The tablets from batch 14 were excluded because after 90 days the tablets were very friable.

Tablets mass

There was significant decrease (p = 0.013, one-way ANOVA) in the mean mass of tablets from batch 33 after 13 weeks when compared with week 0. There was no further decrease in the mean mass of tablets from batch 33, 3 weeks later (total 16 weeks) when compared with week 13 (Figure 4.21, see Table 1.10, Appendix 2). However there was no significant change (p > 0.05, one-way, ANOVA) in the mean mass of tablets from batches 5, 8, 11 and 33 and control tablets, stored at 75% RH and 40°C at the end of the experiments. The decrease in the mean mass for tablets from batches 5, 8 and 33 stored at 75% RH and 40°C after 180 days / 6 weeks ranged between 1.67 mg for batch 8 and 1.10 mg for batch 33 (Figure 4.21, see Table 1.10, Appendix 2) .

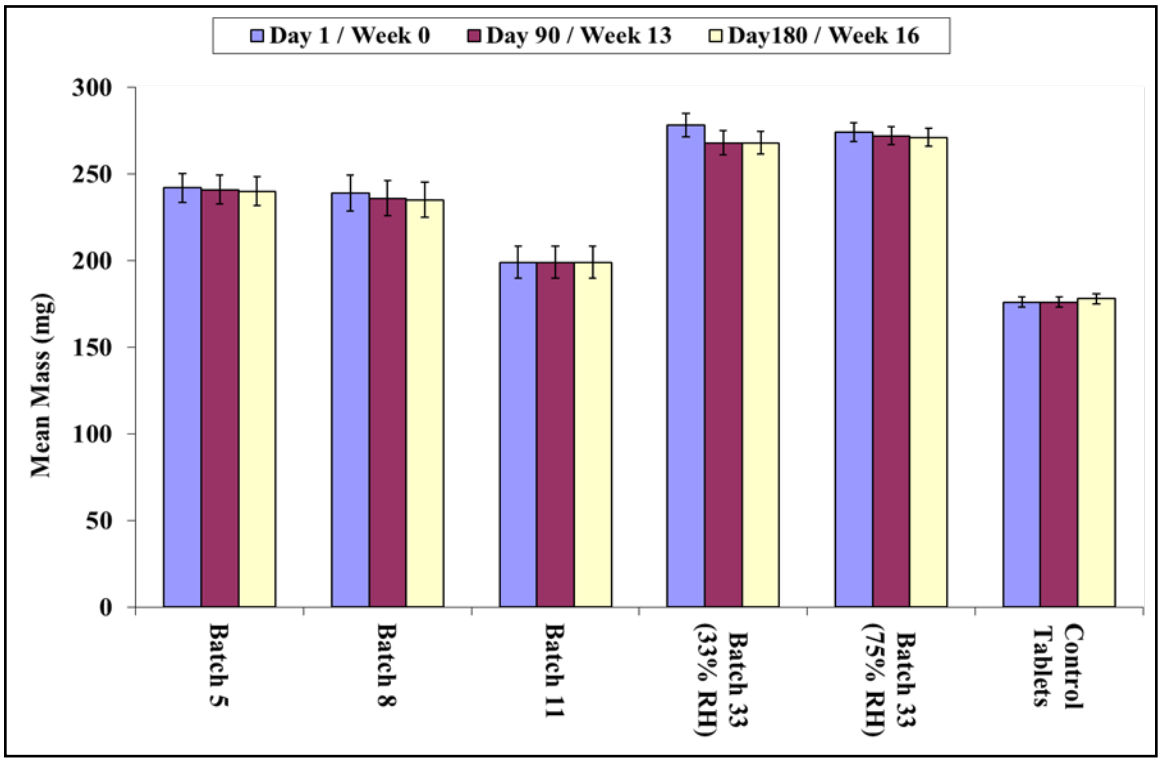


Figure 4.21 Mean mass (mg) of tablets at days 1, 90 and 180 for tablets from batches 5, 8 and 11 stored at 75% RH at 40°C and at weeks 0, 13 and 16 for tablets from batch 33 stored at 33 and 75% RH at 35 and 40°C (Error bars represent the SD of each mean).

Tablets diameter

There was significant increase ($p < 0.001$, one-way ANOVA) in the mean diameter of tablets from batches 5, 8, 11 and 33 which were stored at 75% RH and 40°C after 90 days (batches 5, 8 and 11) and 13 weeks (batch 13) respectively (see Table 4.11, Appendix 2). The increase in the mean diameter of the tablets from batches 5, 8 and 11 was again significant ($p \leq 0.001$, one-way ANOVA) after another 90 days (total 180 days) (Figure 4.22, see Table 1.11, Appendix 2). There was no change in the mean diameter of tablets from batch 33, stored at 75% RH and 40°C, after another 3 weeks (total 16 weeks). The increase in the mean diameter of the tablets from the batches 5, 8 and 11 after 180 days and the tablets from batch 33 after 16 weeks, stored at 75% RH and 40°C, ranged between 3.79% for the tablets from batch 5 and 1.10% for the tablets from batch 33.

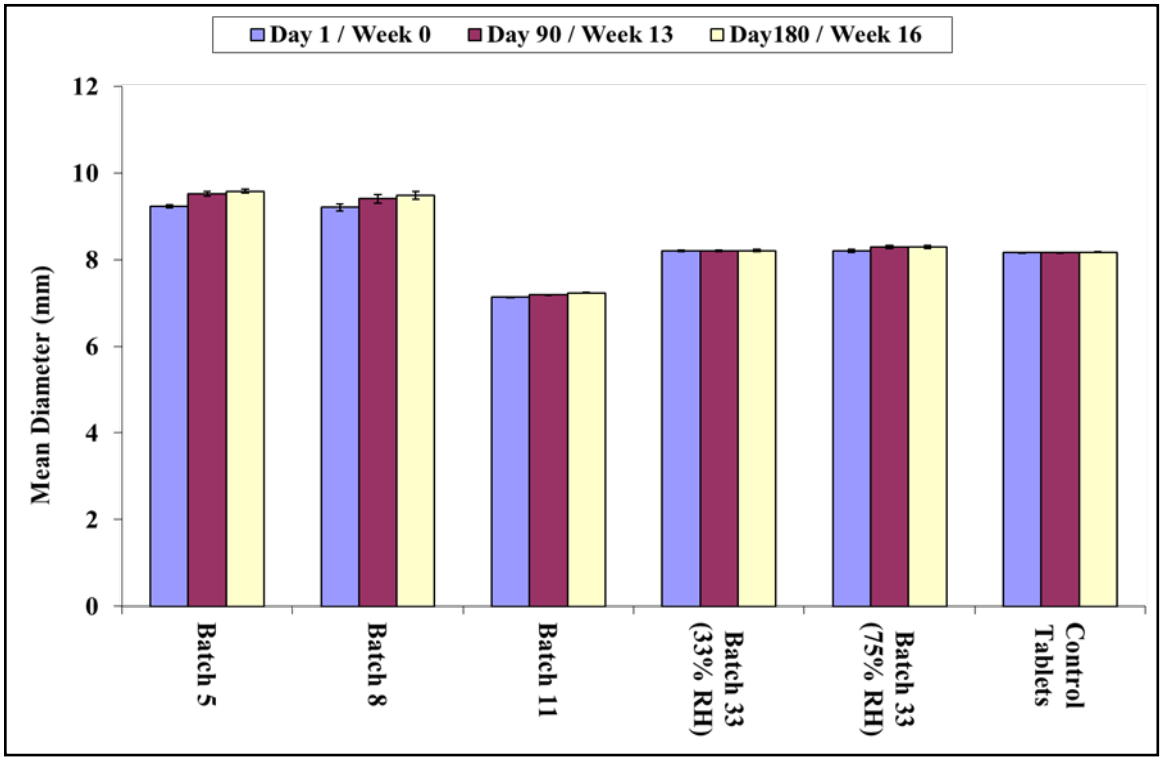


Figure 4.22 Mean diameter (mm) at days 1, 90 and 180 for tablets from batches 5, 8 and 11 stored at 75% RH at 40°C and at weeks 0, 13 and 16 for tablets from batch 33 stored at 33 and 75% RH at 35 and 40°C (Error bars represent the SD of each mean).

There was no significant change ($p > 0.05$, one-way ANOVA) in the mean diameter of tablets from batch 33 stored at 33% RH and 35°C after 16 weeks and the control tablets after 180 days (Figure 4.22, see Table 1.11, Appendix 2).

Tablets thickness

There was significant increase ($p \leq 0.345$, one-way ANOVA) in the mean thickness of tablets from batches 5, 8, 11 and 33 which were stored at 75% RH and 40°C after 90 days (batches 5, 8 and 11) and 13 weeks (batch 13) respectively. The increase in the mean thickness of the tablets from batches 5, 8 and 11 was again significant ($p \leq 0.148$, one-way ANOVA) after another 90 days (total 180 days) (Figure 4.23, see Table 1.12, Appendix 2). There was no significant change ($p > 0.05$, one-way, ANOVA) in the mean thickness of tablets from batch 33, stored at 75% RH and 40°C, after another 3 weeks

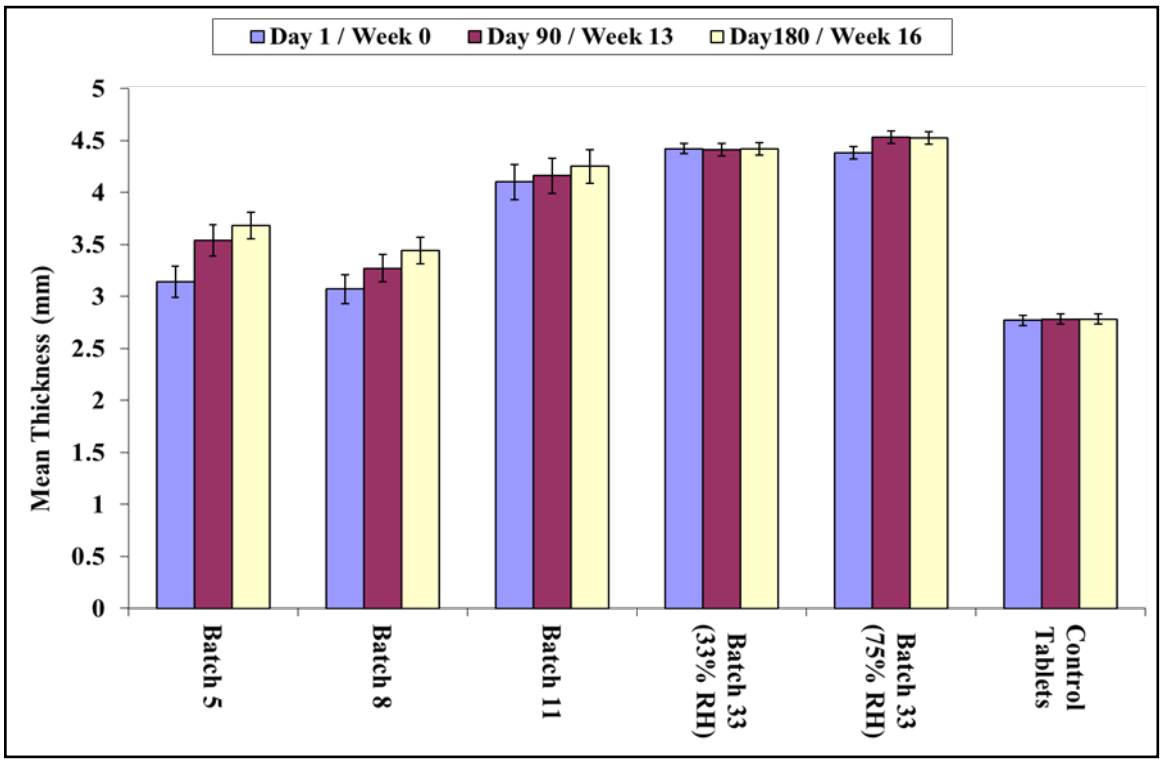


Figure 4.23 Mean thickness (mm) at days 1, 90 and 180 for tablets from batches 5, 8 and 11 stored at 75% RH at 40°C and at weeks 0, 13 and 16 for tablets from batch 33 stored at 33 and 75% RH at 35 and 40°C (Error bars represent the SD of each mean).

(total 16 weeks). The increase in the mean thickness of the tablets from the batches 5, 8 and 11 after 180 days and the tablets from batch 33 after 16 weeks, stored at 75% RH and 40°C, ranged between 17.20% for the tablets from batch 5 to 3.20% for tablets from batch 33. There was no significant change ($p > 0.05$, one-way ANOVA) in the mean thickness of tablets from batch 33 stored at 33% RH and 35°C after 16 weeks and the control tablets after 180 days (Figure 4.23, see Table 1.12, Appendix 2).

Tablets volume

There was significant increase ($p \leq 0.009$, one-way ANOVA) in the mean volume after 90 days for the tablets from batches 5, 8, 11 and 13 weeks for the tablets from batch 33 which were stored at 75% RH and 40°C. The increase in the mean volume of the tablets

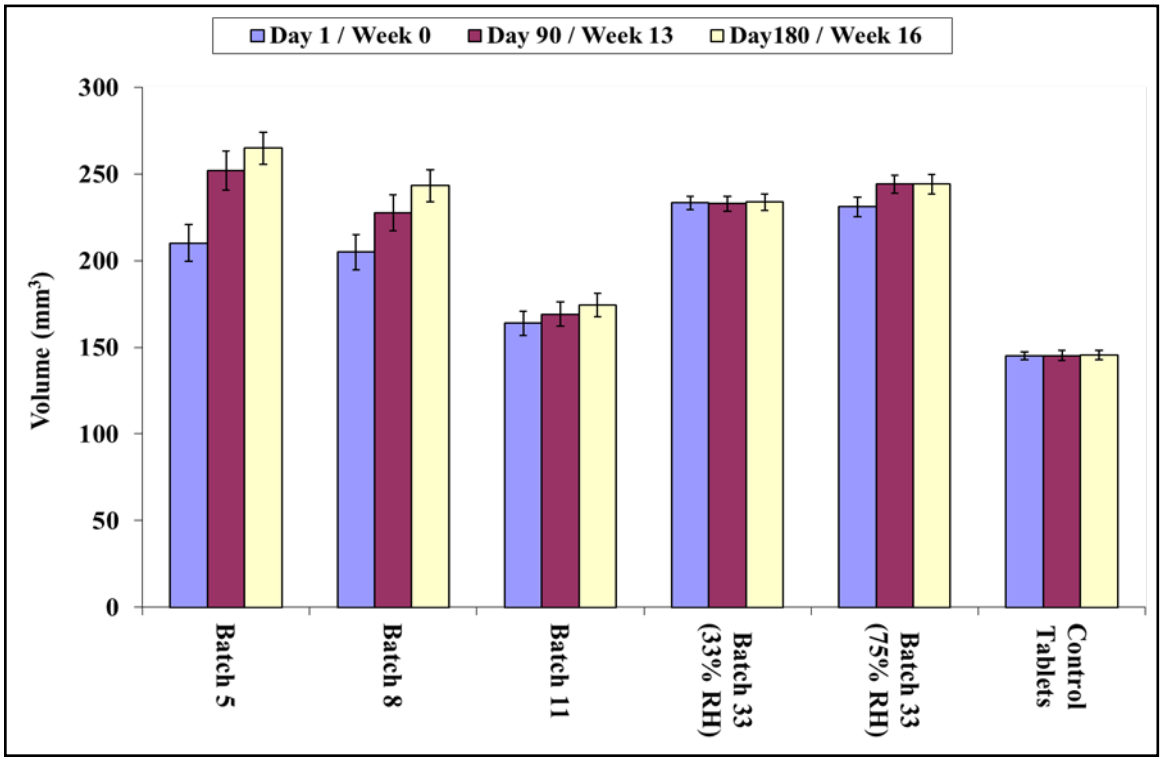


Figure 4.24 Mean volume (mm^3) at days 1, 90 and 180 for tablets from batches 5, 8 and 11 stored at 75% RH at 40°C and at weeks 0, 13 and 16 for tablets from batch 33 stored at 33 and 75% RH at 35 and 40°C (Error bars represent the SD of each mean).

from batches 5, 8 and 11 was again significant ($p \leq 0.013$, one-way ANOVA) after another 90 days (total 180 days) (Figure 4.24, see Table 4.12, Appendix 2). There was

no significant change ($p > 0.05$, one-way ANOVA) in the mean volume of tablets from batch 33, stored at 75% RH and 40°C, after another 3 weeks (total 16 weeks). The increase in the mean volume of the tablets from the batches 5, 8 and 11 after 180 days and the tablets from batch 33 after 16 weeks, stored at 75% RH and 40°C, ranged between 26.12% for the tablets from batch 5 to 5.67% for tablets from batch 11. There was no significant change ($p > 0.05$, one-way ANOVA) in the mean volume of tablets from batch 33 stored at 33% RH and 35°C after 16 weeks and the control tablets after 180 days (Figure 4.24, see Table 4.12, Appendix 2).

Hardness

The hardness was very low (< 20 N) for the tablets from batches 5 and 8 after 180 days and for the tablets from batch 33 after 16 weeks, stored at 75% RH and 40°C. The hardness was greater than 40 N for the tablets from batch 11 and control tablets stored at 75% RH and 40°C after 180 days and for the tablets from batch 33 stored at 33% RH and 35°C after 16 weeks.

4.5 Discussion

The main aim of these experiments was to establish the ideal storage conditions for 'ecstasy' tablets. The determination of the right storage conditions would help forensic laboratories to properly store and preserve the physical features of 'ecstasy' tablets for the eventual use of these features to link or differentiate batches for court, investigative and intelligence purposes.

4.5.1 Experiment A: Photostability stress testing on batches 5, 8, 11 and 14

The surface colours of the four samples of tablets were found to have changed at the end of the photostability stress testing experiment when compared to control tablets. Colour testing using a spectrocolourimeter revealed a very slight discolouration ($\Delta E^* = 0.42$) of the white tablets with the euro logo (batch 11). The same colour tests indicated a very

slight ($\Delta E^* = 0.42$) to $\Delta E^* = 0.54$) colour change in the blue and green tablets (batches 5 and 8 respectively). The change in the colour of the orange tablets with pisces logo (batch 14), which could be seen by the naked eye, was very obvious ($\Delta E = 5.06$). The human eye can perceive a colour change of $\Delta E > 1.5$ [230].

The photostability stress testing experiment have shown that the colour of 'ecstasy' tablets can change if tablets are left exposed to visible and UV light for long periods of time (e.g. this experiment ≈ 39 days - adopted ICH method). If colour is used as one of the physical features to link batches of 'ecstasy' tablets a significant change in the colour would be necessary, as happened with the orange tablets from batch 14, in order for this to be observed by the naked eye. This experiment has also shown that to preserve the colour the tablets should be protected from visible and UV light. Thus if the colour of tablets is to be used for forensic drug intelligence the tablets should be stored in light-resistant containers.

Colour and colour variance are basic features of many 'ecstasy' tables that together with other characteristics, such as shape, mass, diameter and thickness could be used to differentiate or link batches of tablets for intelligence purposes. Colour has often been discarded as a discriminating feature as it is claimed that it is not reliable and it is highly dependent on the operator [181]. The visual observation of colour is subjective and lack precision [230, 256]. In this study the lack in exactness and objectivity in colour assessment was addressed by reflectance spectroscopy using a portable spectrocolourimeter together with the CIELAB colour system to get an exact numerical specification of the human colour vision [230, 256]. Colour vision, which is trichromatic, is calculated by the CIEL^*a^* and b^* (CIELAB) colour space values. Each colour has a unique location in three dimensional space as defined by its Cartesian coordinates which are the L^* (brightness axis), a^* (red-green axis) and b^* (yellow-blue axis). Spectrophotometric techniques together with CIELAB colour values have already been described in the literature, and have been used during stability testing of pharmaceutical products [256] and in the field of food and dyes industry [256]. However, as far as is known, there has never been any study on the application of

instrumental evaluation of colour of 'ecstasy' tablets using the CIELAB colour system for intelligence purposes.

The photostability experiment has shown that the use of a portable spectrocolourimeter together with CIELAB colour system was a quick, simple and good method that could be used to differentiate or link batches of 'ecstasy' tablets by their colour, including white tablets. Using this method it was possible to differentiate between the four batches (5, 8, 11 and 14) of 'ecstasy' tablets used as controls and also to differentiate between the tablets from the same batch at the end of experiment, that is between the control and exposed tablets. The results obtained from the experiment were very promising for differentiating between batches of 'ecstasy' tablets, and it would be worthwhile that further experiments be performed.

If this method is to be used for drug forensic purposes a sample of about ten 'ecstasy' tablets would be needed. The colour of the tablets, which should always be taken at random from the batch, would be measured by a portable spectrocolourimeter using the L^*, a^* and b^* values. Batches of 'ecstasy' tablets having similar colour could be differentiated or linked using one-way ANOVA for the L^*, a^* and b^* values. However the L^*, a^* and b^* values are instrument dependent and thus the CIELAB values could only be compared when measurements are conducted with similar or correlated instruments [256].

4.5.2 Accelerated and stability experiments on batches 5, 8, 11, 14 and 33

When 'ecstasy' tablets were subjected to varying conditions of RHs and temperatures the main discriminative measurable features, mass, diameter and thickness were found to be affected. Other calculated and measured features such as the volume, hardness, friability and disintegration were also found to be affected.

4.5.2.1 Experiment B: Accelerated storage testing on batches 5, 8, 11 and 14

The high RH (75%) and temperature of 40°C that were used during the accelerated storage experiment did not seem to have had any significant effect ($p > 0.05$, one-way ANOVA) on the mean mass of tablets from batches 5, 8 and 11, after 180 days. However, the mean mass of the sample of tablets from batch 14 had significantly decreased ($p = 0.013$, one-way ANOVA) after 90 days from the start of the experiment. The decrease in the mean mass of the tablets from batch 14 was probably caused by a high increase in the friability. The physical properties of the tablets during storage at constant RH and temperature are dependent on the equilibrium moisture content (EMC). The EMC is the amount of moisture which a tablet contains when it reaches equilibrium with the surrounding environment [257]. When the RH is high, such as was the RH (75%) used in the accelerated storage experiment, the tablets would reach the critical moisture content (CMC), which is the moisture at which the physical features, such as the friability and hardness, would start to deteriorate. Thus if the increase in friability of the tablets from batch 14 had occurred during the clandestine storage, the tablets would not have withstanded the rough handling and transportation during trafficking.

The diameter and thickness, considered the most reliable features for intelligence purposes, of the 'ecstasy' tablets used in the experiment had increased significantly ($p < 0.05$, one-way ANOVA) when subjected to 75% RH and 40°C after 180 days. The increase in the diameter and thickness caused a significant increase in the volume ($p \leq 0.013$, one-way ANOVA) and the tablets to swell. The other three physical features, the hardness, friability and disintegration, were also affected by the accelerated storage conditions. After 180 days the hardness of the tablets from batches 5 and 8 could not be measured because the tablets were very friable, the tablets from batch 14 had disintegrated, and the hardness of the tablets from batch 11 had significantly decreased ($p < 0.001$, one-way ANOVA). Moreover, with the exception for the tablets from batch 11 the friability of the tablets from batches 5 and 8 had increased while the disintegration had decreased after 180 days. This indicated that the RH and the temperature used in the accelerated storage testing had adversely affected the measurable features of the tablets. The changes noticed with the 'ecstasy' tablets have also been noticed with pharmaceutical tablets prepared in the laboratory when these were stored at high RH

(75%) and room temperature [258]. Moreover, similar to 'ecstasy' tablets extensive volume expansion was also observed when laboratory prepared pharmaceutical tablets were exposed to 75% RH [258]. The extensive changes noticed in the examined 'ecstasy' tablets, also noticed with pharmaceutical tablets when stored at 75% RH, occur when the tablets reach the CMC deteriorating in the process the physical features such as friability and hardness [254].

4.5.2.2. Experiment C: Stability testing: varying conditions of RHs and temperatures

At the start of the experiment it was noted that the mass of the individual tablets, used for the 8 samples for the stability experiment, varied from the mean of 274 mg by - 4.40 to + 4.00%. The tablets prior to the experiment were kept in an air conditioned room at 25% RH and a temperature of 25 $\pm$ 2°C. From the individual tablet mass measurements it transpired that two sample populations were present within batch 33 containing tablets with the versace logo (Figure 4.3 above). There are several possible explanations for this observation. Studies to investigate the mass variation of pharmaceutical tablets produced by single punch tabletting machines, also used for 'ecstasy' tablets production [137], have attributed the variation in the mass to the non uniform filling of the dies. Moreover, mass variation could also be caused by the use of old punches and dies and by the multiplicity of punches and dies used during tablet production by multiple-station tabletting machines, which are also used for 'ecstasy' tablet production [259]. Studies conducted on pharmaceutical standard tablets have shown that if one of the lower punches or both upper and lower punches are short by 0.05 mm the tablet will be about 0.4 % overweight [259]. Since the RSDs of the diameter and thickness of the examined 'ecstasy' tablets were very low (0.24 and 1.59% respectively) it could be postulated that tablets with a mean mass of 268 mg, might have been produced together with the overweight tablets, mean mass of 280 mg, on the same tabletting machine.

There was no statistical difference in the mean mass of each sample of tablets stored at 33 and 75% RH and all temperatures, with the exception of the sample stored at 33% RH and 35°C, at the first and second week when compared with week 0. Nevertheless, it was

noticed that during the first week the mean mass of the tablets in three out of the four samples stored at 33% RH and 5, 25 and 35°C had decreased, while the mean mass of the tablets in the four samples stored at 75% RH regardless of temperature had increased. Moreover, it transpired that after 1 week the greatest decrease in the mean mass occurred when the tablets were stored at 33% RH and 35°C (decrease of 7.3 mg), while the greatest increase in the mean mass occurred when the tablets were stored at 75% RH and 15°C (increase of 4.2 mg) . These observed results were similar to results obtained in a study conducted on pharmaceutical tablets in blister packs where the mass of tablets had increased (≈ 2 mg at 75% RH and 21 - 22°C, Amidon and Middleton (1998)) [260]. It was reported that when tablets were exposed to humidities greater than 60% the mass tended to increase, but decreased when exposed to lower humidities (≈ 3.5 mg at 25% RH and 21 - 22°C; this study decrease of 2.5 mg at 33% RH and 25°C) [260]. Amidon and Middleton [260] did not statistically evaluate the increase and decrease in mass of tablets. However, after 16 weeks there was no statistical difference in the mean mass of 'ecstasy' tablets in seven out of the eight separate samples stored at 33 and 75% RH, regardless of temperature, when compared to week 0. The only exception was the sample of tablets stored at 33% RH at 35°C where after 16 weeks the mean mass had statistically decreased when compared with week 0.

The mean diameter of tablets significantly increased at 33% RH and 5°C, but otherwise the mean diameter and thickness of the tablets remained unaffected after 16 weeks, regardless of temperature. However, the mean diameter and thickness of the tablets stored at 75% RH were significantly increased over 16 weeks regardless of temperature when compared to tablets stored at 33% RH. This finding confirmed the results of the accelerated storage experiment where the mean diameter and thickness of tablets stored at 75% RH and at 40°C had increased after 90 and 180 days respectively. Moreover, on week 16 there was no statistical significant difference between the mean thickness of the tablets in the 4 separate samples stored at 75% RH and all temperatures but there was statistical difference between the mean diameters of the tablets.

Considering only the RH both the mean diameter and mean thickness of the total tablets in the 4 samples stored at 75% RH had increased after 16 weeks (mean diameter

increased from 8.19 to 8.27 mm, an increase of 0.98%, and the mean thickness increased from 4.39 to 4.50 mm, an increase of 2.51%). Again considering only the RH there was no significant difference ($p > 0.05$, one-way ANOVA) at week 0 in the mean diameter of the total tablets in the 4 samples stored at 75% RH when compared with the mean diameter of the total tablets in the 4 samples stored at 33% RH. However, there was a significant increase ($p < 0.05$, one-way ANOVA) in the mean diameter of the total tablets stored at 75% RH when compared with the mean diameter of the total tablets stored at 33% RH for all weeks 1 to 16. Moreover, when considering only the RH there was no significant difference ($p > 0.05$, one-way ANOVA) at week 0 in the mean thickness of the total tablets in the 4 samples stored at 75% RH when compared with the mean diameter of the total tablets in the 4 samples stored at 33% RH. There was but a significant increase ($p < 0.05$, one-way ANOVA) in the mean thickness of the total tablets in the 4 samples stored at 75% RH when compared with the mean thickness for the total tablets in the 4 samples stored at 33% RH for all weeks 1 to 16.

As far as it is known, this was the first study which investigated the stability of the measurable physical features of 'ecstasy' tablets at different RHs and temperatures. Changes in the measurable features of 'ecstasy' tablets caused by changes in the environmental conditions during storage of the tablets could affect the use of these features for forensic drug intelligence purposes. The overall overview of the stability study was that at 75% RH there was a significant increase ($p < 0.05$, one-way ANOVA) in the mean diameter, thickness and volume of the tablets with duration of storage regardless of the temperature. However, for the tablets at 75% RH the greatest increase in the mean diameter occurred at 15 and 40°C respectively. The greatest increase for the mean thickness and volume occurred at 40 and 15°C respectively. The effect of the increase in the volume of the tablets was that of swelling. After 16 weeks the swelling of the tablets stored at 75% RH and all temperatures caused a statistical decrease in the hardness of the tablets. The swelling of tablets observed at 75% RH was in line with other scientific studies on pharmaceutical tablets stored at fairly high to high RHs (65-95%) [252, 258, 260] were the decrease in hardness was followed by increase in friability [261]. It has been suggested that the increased adsorption at high water vapour by tablets

can disturb the interparticulate bonds in the tablet thus decreasing the hardness and friability [258]. However, the adsorption of moisture by the tablet also depends on the excipients and the hardness of the tablet [261]. The harder the tablets the less moisture it absorbs [261]. The hardness of the 'ecstasy' tablets used in this study was relatively low (47.00 N mean hardness) at the start of the experiment, further explaining the reason for the significant decrease in the hardness of the tablets stored at 75% RH at all temperatures after 16 weeks.

In stability studies in the literature the major importance is generally given to the effect of RH on the physical features of pharmaceutical tablets and the effect of temperature is not much discussed [253, 254, 258, 260, 261]. However, from the results of this stability experiment it transpired that the physical features of samples of 'ecstasy' tablets stored at 75% RH and different temperatures were affected differently. When comparing the results from the stability experiment of 4 samples of tablets stored at 75% RH but at different temperatures (5, 15, 25 and 40ºC) it was noted that the most significant changes in the physical features had occurred when the tablets were stored at a temperature of 40ºC. The mean hardness of the tablets stored at 75% RH and 40ºC had decreased much more markedly than the mean hardness for the samples stored at the same RH but lower temperatures (5, 15 and 25ºC) after 16 weeks. Similar results were obtained by Ahmad and Shaikh (1994) where after 5 months the hardness of 5 samples of tablets, stored at 75% RH, had decreased when the temperature of the samples was increased from 25 to 45ºC (the decrease in hardness ranged between 0.52 to 39.13%) [254]. The significant changes in the physical features of tablets at 40ºC when compared to tablets stored at the other temperatures (5, 15 and 25ºC) and 75% RH was caused by the increased amount of water in the air at 40ºC [262]. The increase in the temperature at a particular RH increases the water in the air and as a result the tablets would take up water in a shorter time from the surrounding air [262] (absolute humidity at 75% RH - 40ºC is 36.0 g m^{-3} compared with 14.9 g m^{-3} at 25ºC).

4.5.3. Difference and changes in the measurable features of 'ecstasy' tablets

Tablets exposed to high RH (75%), during the accelerated storage and stability experiments, had a marked effect on the measurable features diameter, thickness, volume, hardness and friability of the tablets. The high RH (75%) used in the two experiments was the main cause for the significant increase in the measurable features of the tablets when compared to tablets stored at 33% RH regardless of temperature. If a hypothetical forensic case scenario is considered where two batches of 'ecstasy' tablets with similar visual features and stored under different environmental condition are seized, then the implications from the obtained results in these studies could be very important. If these batches are seized on different occasions they might have different measurable features making it difficult to link the batches. The batch stored at high RH ($\geq$ 60%) might have reduced hardness, high friability or could even disintegrate as happened with batch 14 in the accelerated storage experiment. This would make it very difficult or even impossible to measure the physical features of the tablets if these are needed for a court case or to be used for forensic drug intelligence purposes.

It could be speculated that at the end of the accelerated storage and stability experiments the increase in diameter and thickness of the tablets stored at 75% RH corresponded to the EMC [262]. During the process the tablets had swelled causing a change in volume, a feature that was noticed with tablets from batches 5, 8, 11 and 33. It could also be hypothesised that the lack of packing material for the 'ecstasy' tablets during the accelerated storage and stability experiments could have caused the tablets to reach the EMC more quickly thus causing the tablets to swell. As for the tablets from batch 14, these had reached the CMC during the accelerated storage and disintegrated at about 170 days

Another reason for the significant change in the physical features of the tablets at high RH could have been due to the added excipients. Lactose, which is the most widely used excipient in legal tablet formulations [205], was reported to be one of the most frequently used excipient in 'ecstasy' tablets [263]. Placebo tablets that were prepared by direct compression using lactose have been reported to swell considerably when stored at 85% RH and 37°C [253]. In this study the swelling of tablets was mostly noted when tablets

were stored at 75% RH regardless of temperature, where the significant increase in mean volume ranged between 5.67% (at 40°C) and 3.19% (at 25° C). Moreover, there was no significant swelling for tablets stored at 33% RH. The results obtained in this study for the increase in the mean volume of 'ecstasy' tablets stored at 75% RH were fairly similar to results reported by Sangekar et al. [253] for tablets with lactose excipient and starch disintegrant (0% for tablets at 25°C and 43% RH and an increase of 4.97 – 5.67% in volume for tablets at 25°C and 75% RH [253]).

The hardness of a tablet provides a measure of the bonding potential of the materials concerned while the friability is a measure of the loss in mass of the tablets due to abrasion or when the tablets attain the CMC and starts to deteriorate. The resistance of tablets to capping, abrasion or breakage under conditions of transportation and handling before usage depends on hardness [264], which is closely related to friability. Also the moisture adsorptive capacity of a compressed tablet is generally considered to be dependent on the hardness, that is the harder the tablet the less moisture it absorbs [261]. Both the friability and hardness are dependent on the RH of the environment which would affect both the rate and the amount of water uptake by the tablets during storage [265]. The interparticulate bonds in the tablets may be disrupted by the moisture [252] if the RH in the environment is high. It could be speculated that the moisture absorption for the tablets from batches 5 and 8 after 180 days when stored at 75% RH and 40°C had affected the interparitculate bonds thus causing a high increase in the friability and a statistical significant decrease in the hardness of the tablets. Similar changes were observed at 75% RH during the stability experiment where the friability had increased and hardness decreased for batch 33 (versace logo) regardless of temperature.

The results have shown that the visible and UV light together with high RHs and temperatures, which could change the physical features of tablets, play an important role in the storage of 'ecstasy' tablets. In cases of seizure of a number of similar or different batches of 'ecstasy' tablets it is normally assumed that the batches were stored under similar conditions of RH and temperatures. Friability testing, which is quick and easy to perform, could be used to determine whether the tablets were stored under similar

conditions. Friability is normally affected by the moisture content in the tablets [254]. As the moisture increases, the interparticulate bonds start to be disrupted, thus increasing the porosity and decreasing the strength of the tablets causing the friability to increase [254]. Difference in the friability (> 0.5%) of batches of 'ecstasy' tablets having similar visual features but seized on different occasions could be an indication that the tablets were stored under dissimilar environmental conditions. Different environmental conditions could cause changes in the measurable feature, such as mass, diameter, thickness, volume, hardness, friability and disintegration rates of similar batches of 'ecstasy' tablets. Thus, if the measurable features of 'ecstasy' tablets are to be used for forensic intelligence purposes to link batches seized on different occasions it would be helpful for officers to first measure the friability of the separate batches to make sure that the batches were not affected by dissimilar environmental conditions. From the accelerated storage and stability experiments it was noted that the most significant changes in the physical features of 'ecstasy' tablets had occurred when the tablets were stored at 75% RH and 40°C when compared with samples stored at temperatures of 5, 15 and 25°C. However, from the stability experiment it had also transpired that the least change in the measurable features had occurred when tablets were stored at 33% RH and at temperatures between 15 and 35°C. Thus it is recommended that batches of 'ecstasy' tablets kept at different laboratories in order to ensure interlab comparability should be stored at low RH (≈ 25%) and temperature (≈ 25 °C) and away from sunlight if the measurable features are to be used for intelligence purposes.

Chapter 5

CHEMICAL CHARACTERISATION OF 'ECSTASY' TABLETS

The chemical characterisation of 'ecstasy' tablets can help link or differentiate between batches of tablets from different seizures irrespective of their physical features. Moreover, the impurities detected in MDMA tablets can serve to act as chemical markers from which the synthetic route could possibly be determined.

5.1 Introduction

The misuse of the amphetamine and amphetamine-type stimulants, such as MA and MDMA has been widely reported with these drugs being consumed worldwide. Typical of the way in which the market for these substances has emerged and remained one step ahead of the authorities has been the manufacture of designer drugs derived from amphetamine. Over the years analogues of amphetamine, such as 4-methoxyamphetamine and 4-methylthioamphetamine [266] have been identified in tablets used by clubbers have been linked to overdose and fatalities [267, 268].

Ecstasy was the street name which was designated to MDMA, but since 2005 some of these tablets started to contain other new psychoactive substances [269]. In addition to the classic empathogens which became scheduled, to evade the law new designer drugs containing the piperazine moiety, such as the 1-aryl-piperazine compounds, known to be psychoactive in man [270] have been identified. One such "family" of drugs, the 1-aryl-piperazines (e.g. BZP drug), has been launched on the grey market by means of the internet in most European drug markets as new designer drugs [270].

These piperazine derived drugs were sold mainly as 'ecstasy' tablets but also in powder or liquid forms [271]. In some seizures of tablets presumed to be ecstasy no MDMA or psychoactive substances was detected [212].

However, ‘ecstasy’ production, which mainly consists of two stages (266) the synthesis of the psychoactive substance/s and (2) tabletting, may be carried out at different locations to avoid detection [269]. Thus it could be speculated that ‘ecstasy’ tablets with the same physical features could have different chemical characteristics. Therefore in addition to physical characterisation of ‘ecstasy’ tablets, profiling of the chemical composition may be valuable for drug intelligence purposes to further differentiate or link batches from different seizures.

The study to investigate the chemical characterisation of batches of ‘ecstasy’ tablet seized over a five year period (2006 – 2011) had several different experiments:

Experiment A: The detection of psychoactive substances (if any) in ‘ecstasy’ tablets from 45 batches using routine colour, TLC, GC-MS and quantification of the active ingredients; the experiment also tested the ***hypothesis (2)*** that:

Seized ‘ecstasy’ tablets will contain MDMA as the predominant psychoactive substance.

Experiment B: The specific identification and the percentage isomeric content of MDMA-only tablets; the experiment also tested the ***hypothesis (3)*** that:

The enantiomeric ratio of MDMA tablets will be 50:50 in llicit tablets.

Experiment C: The organic impurity profiles of MDMA-only tablets;

Experiment D: The determination of the major excipients of ‘ecstasy’ tablets using FTIR transmittance spectroscopy;

Experiment E: The elemental profiling of ‘ecstasy’ tablets using SEM/EDX analyses.

These experiments also tested the ***hypothesis (4)*** that:

The chemical composition of different batches of ‘ecstasy’ tablets seized on different occasions in Malta during 2006 – 2011 will be significantly different from each other.

5.2 Methods

In this study the same 45 batches of 'ecstasy' tablets used for the physical characterisation (Chapter 3) were used for chemical characterisation (Table 5.1).

Table 5.1 The 45 batches of 'ecstasy' tablets that were used for the chemical characterisation experiments (Exp.) to determine (A) the psychoactive substances, (B) percentage isomeric content and (C) organic impurity profiles of MDMA-only tablets, (D) major excipients and (E) elemental profiling of 'ecstasy' tablets.

Batch No.	Exp. A - E	Tablet	Batch No.	Exp. A - E	Tablet	Batch No.	Exp. A - E	Tablet
1	A		16	A, D, E		31	A, D, E	
2	A, E		17	A – E		32	A, D, E	
3	A		18	A – E		33	A – E	
4	A, D, E		19	A – E		34	A, D, E	
5	A – E		20	A – E		35	A, D, E	
6	A, D, E		21	A – E		36	A, D, E	
7	A, D, E		22	A – E		37	A, D, E	
8	A, E		23	A – E		38	A, D, E	
9	A, D, E		24	A – E		39	A, D, E	
10	A, D, E		25	A – E		40	A – E	
11	A – E		26	A – E		41	A – E	
12	A, D, E		27	A – E		42	A, D, E	
13	A, D, E		28	A – E		43	A, D, E	
14	A – E		29	A – E		44	A – E	
15	A, D, E		30	A, D, E		45	A – E	

5.2.1 Experiment A: Detection and quantification of psychoactive substances in batches of tablets

5.2.1.1 Colour tests and TLC

Colour tests of tablets from all batches were conducted on small amounts (~1-2 mg) of crushed 'ecstasy' tablets using Marquis, Simons's, Chen's and Scott's Reagents [210], while TLC was done on samples taken from the remaining tablets samples as described in the methodology, Section 2.2.3.1, for colour tests and for TLC. A negative control and positive controls of amphetamine, MDMA, MDA, BZP, mCPP, caffeine, methandrostenolone and cocaine were used during these analyses.

Although when using the combination of colour tests and TLC it was possible to discriminate between MDMA and the other reference standards used (MDA, amphetamine, BZP, mCPP, caffeine and cocaine) due to the number of amphetamine type stimulants available, it was necessary to use GC-MS analyses to confirm the results.

5.2.1.2 Determination of the psychoactive substances in 'ecstasy' tablets

The determination of the major active ingredients in 'ecstasy' tablets was based on the liquid extraction of samples of crushed tablets taken at random from the 45 batches (one tablet from each batch), followed by GC-MS analyses as described in the methodology Section 2.2.3.1.

5.2.1.3 Quantification of active ingredients in 'ecstasy' tablets

Quantification of all 'ecstasy' tablets containing only one active ingredient was carried out by UV spectrophotometry. The wavelength of the principal peaks for each single active ingredient found in the tablets together with the corresponding absorptivity was noted. The quantity of the drug in each tablet was calculated from a standard curve for the particular psychoactive substance.

5.2.2 Experiment B: Specific identification and percentage isomeric content of MDMA-only tablets

Samples from batches of tablets which gave positive results for MDMA (batches 5, 8, 11, 14, 17-29, 33, 40, 41, 44 and 45) were derivatised using TFAA to confirm the presence of MDMA in the tablets. The same derivatised samples were used to determine the enantiomeric composition of the MDMA in the tablets. The samples were analysed by GC-MS using an Rt-βDEXcst-TM capillary column. The method was validated for the derivatised single enantiomer and racemic MDMA as described in Section 2.2.3.2.

5.2.3 Experiment C: Organic impurity profiling of MDMA-only tablets

The organic impurity profiling analyses (Section 2.2.3.3), which were carried out on tablets from batches indicated in Section 5.2.2 (see Table 5.1 above), were conducted to determine the possible synthetic routes of MDMA in the tablets. The GC-MS profiles were treated like fingerprints to link / discriminate tablets taken from the different batches. The profiling method, which was adapted from Gimeno et al. [121, 205], consisted of extraction of MDMA-only tablets using liquid-liquid extraction under basic conditions with subsequent GC-MS analyses.

5.2.4 Experiment D: Determination of major excipients of 'ecstasy' tablets

To determine the major excipients FTIR spectroscopy by transmission was used with the KBr disk technique (Section2.2.3.4). The KBr disk spectra, which were also used for characterisation of the tablets, were recorded between 4000 and 500 cm^{-1}, by averaging 32 scans for each spectrum at a resolution of 4 cm^{-1}. The analyses were conducted on single tablets from batches 4-18, 21-23, 25, 26, 28, 29, 31-41, 44 and 45 (35 tablets), as indicated in Table 5.1 above

5.2.5 Experiment E: Elemental profiling of 'ecstasy' tablets

As described in Section 2.2.3.5 tablet samples were analysed using SEM/EDX, which is a 'surface' technique with a penetration depth of about 2-5 μm, that allow the elemental characterisation of minor and major elements. The SEM/EDX method was used for the

analyses of compounds containing elements with atomic number ≥ 11, other than carbon and oxygen, found on the surface of tablets at a concentration of at least 0.1% by weight. The signals used for capturing the image of the tablets were secondary electrons (SEI) and backscatter electrons – shadow.

Analyses were conducted on a total of 37 individual tablets that were taken from the batches 2, 4-18, 21, 23, 24, 26-41, 44 and 45 (37 tablets) as described in Table 5.1 above. The tablets were each mounted on a SEM sample stub using double-sided carbon adhesive tape and coated with a thin carbon layer in a sputter coater (Emiscope, UK) in order to obtain a conductive surface. The sample tablets were observed from the SEM, with elemental compositions being analyzed by EDX detector as described in Section 2.2.3.5.

5.3 Results

5.3.1 Experiment A: Detection and quantification of psychoactive substances in batches of tablets

5.3.1.1 Colour tests and TLC

Tablets from batch 1 gave an orange → brown with Marquis reagent, indicative of amphetamine type substance and the tablets from batches 2 and 3 gave a blue colour with Simon's reagent, indicative of secondary amines. The tablets from batches 5-29, 33, 40, 41, 44 and 45 gave a positive colourimetric tests results, giving a dark-blue black colour with Marquis reagent and a deep blue colour with Simon's reagent. Tablets from batch 30 gave an orange colour with Marquis reagent. None of the tablets gave a colour change with the Scott's and Chen's reagents. Moreover the tablets from batches 4, 31, 32, 34-39, 42 and 43 did not give any colour change with Marquis and Simon's reagents (Table 5.2).

The TLC tests carried out on extracts from the tablets from batches 1, 5 - 16, 17-29, 33,

40, 41, 44 and 45 gave a blue spot with acidified iodoplatinate spray and an orange red spot with fast black K spray (tablets in batch 1 - *Rf* 0.87 $\pm$ 0.01 and batches 5-16, 17-29, 33, 40, 41, 44 and 45 - *Rf* 0.39 $\pm$ 0.01) (Table 5.3). The tablets from batches 2–4, 31, 32

Table 5.2 The colour test results of 'ecstasy' tablets from the 45 batches and controls.

Batches	Marquis	Simon's	Scott's	Chen's
1	orange → brown	NR	NR	NR
2 and 3	NR	blue	NR	NR
5-29, 33, 40, 41, 44 and 45	dark blue-black	deep blue	NR	NR
30	orange	NR	NR	NR
4, 31-32, 34-39, 42 and 43	NR	NR	NR	NR
Controls				
Amphetamine	orange → brown	NR	NR	NR
MDMA	dark blue-black	deep blue	NR	NR
MDA	dark blue-black	NR	NR	NR
BZP	NR	blue	NR	NR
mCPP	NR	NR	NR	NR
Caffeine	NR	NR	NR	NR
Methandrostenolone	orange	NR	NR	NR
Cocaine	NR	NR	blue ppt., then pink, and pink over blue	NR
Negative	NR	NR	NR	NR

Key: NR = no reaction

34 - 39 gave a blue coloured spot with acidified iodoplatinate spray (tablets from batches 2 and 3 - *Rf* 0.27; batches 4, 31 and 32 - *Rf* 0.29 and batches 34-39 - *Rf* 0.52 $\pm$ 0.01) and no colour with fast black K spray. Extracts from tablets from batches 30, 42 and 43 gave no colour spots with the used visualization sprays.

5.3.1.2 Organic profiling of 'ecstasy' tablets

All the tablets were initially analysed by GC-MS for the compound MDMA. Thirty batches (66.7%) (batches 5-29, 33, 40, 41, 44 and 45) were found to contain only MDMA (Figure 5.1). The remaining batches (batches 1-4, 30- 32, 34-39, 42 and 43) were tested for other psychoactive substances including DPIA, BZP, mCPP and caffeine.

Table 5.3 The TLC test results of 'ecstasy' tablets from the 45 batches and controls.

Batches	***Rf*[1] mean (SD)**	**Acidified iodoplatinate**	**Fast Black K**
1	0.34 (0.01)	blue	orange red
2 and 3	0.27 (0.01)	blue	NR
4, 31 and 32	0.29 (0.01)	blue	NR
5 - 29, 33, 40, 41, 44 and 45	0.39 (0.01)	blue	orange red
34 – 39	0.52 (0.01)	blue	NR
30, 42 and 43	/	NR	NR
Controls			
Amphetamine	0.51	blue	purple
MDMA	0.39	blue	orange red
MDA	0.35	blue	violet
BZP	0.26	blue	NR
mCPP	0.30	blue	NR
Caffeine	0.53	blue	NR
Methandrostenolone	/	NR	NR
Cocaine	0.60	blue	NR
Negative	/	NR	NR

Key: SD – Standard deviation; NR - no reaction
[1]Rf is the retardation factor

The tablets from batch one were further analysed for the presence of amphetamine. Although with the GC-MS system used it was possible to obtain a molecular ion and

individual fragments for amphetamine, if the compound was present in very low concentration it would not have produced molecular ions. Moreover, the mass spectrum

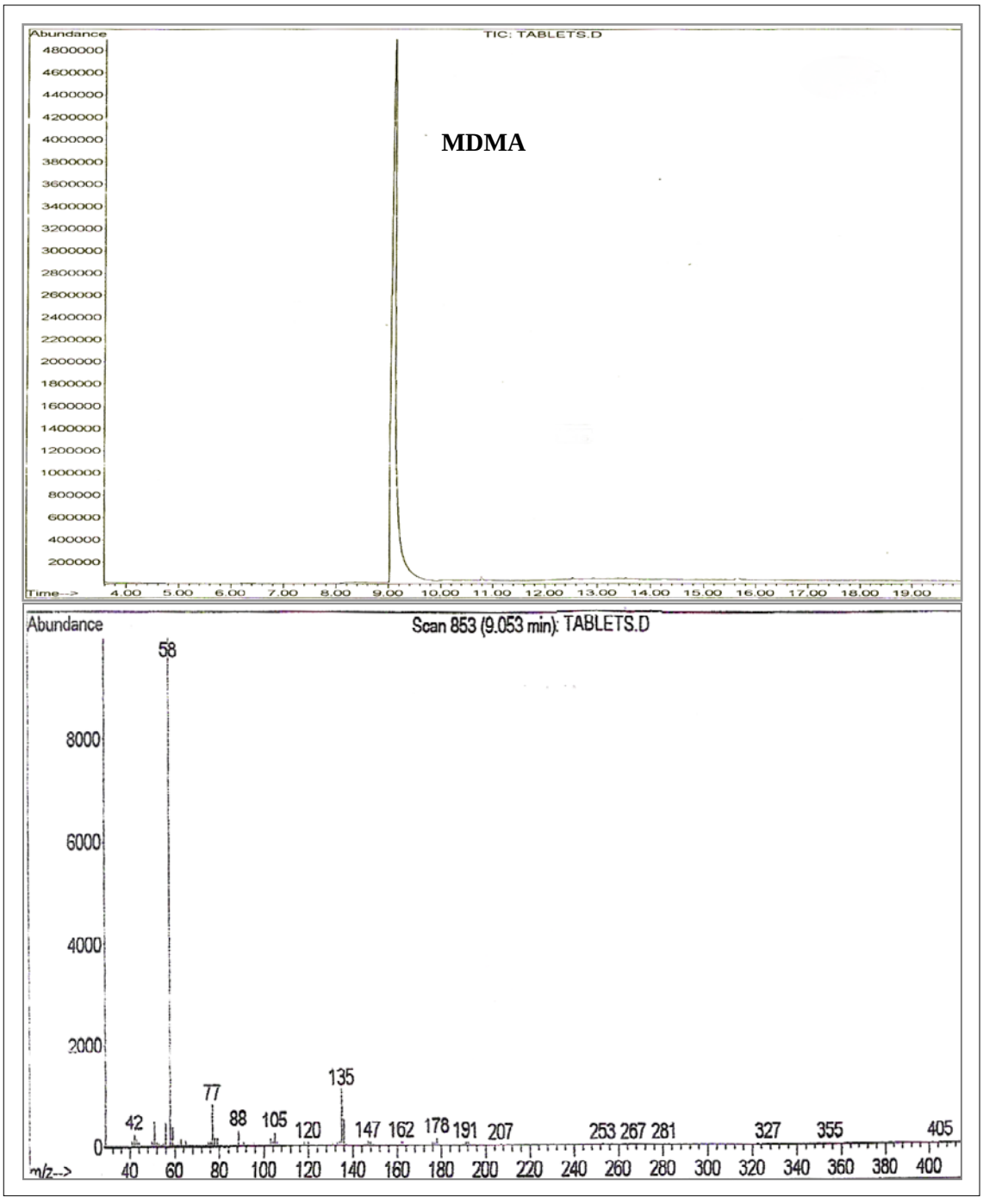


Figure 5.1 The top trace is the total ion chromatogram (TIC) of MDMA at retention time (*Rt*) of 9.05 min for a pink tablet from batch 44 (crocodile (lacoste) logo) and the lower trace is the EI mass spectrum of MDMA from the tablet (58 base peak with ions m/z 135, 77 and 136).

of non-derivatised amphetamine is dominated by fragment ions of low molecular weight which are not particularly characteristic. Although the molecular ion is more characteristic, it is possible that if amphetamine was present in samples at very low concentration, the molecular ion would not be visible in the mass spectrum. Hence to test for the presence of amphetamine, extracts from the tablets were initially derivatised using carbon disulphide (CS_2) (Figure 5.2) and then analysed by GC-MS. Derivatisation of amphetamine with CS_2 not only produce a more characteristic mass spectrum but it also reduces the polarity of the drug, thereby improving chromatographic behavior and

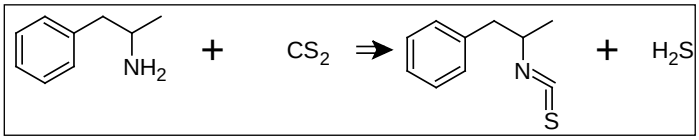


Figure 5.2 Derivatisation of amphetamine.

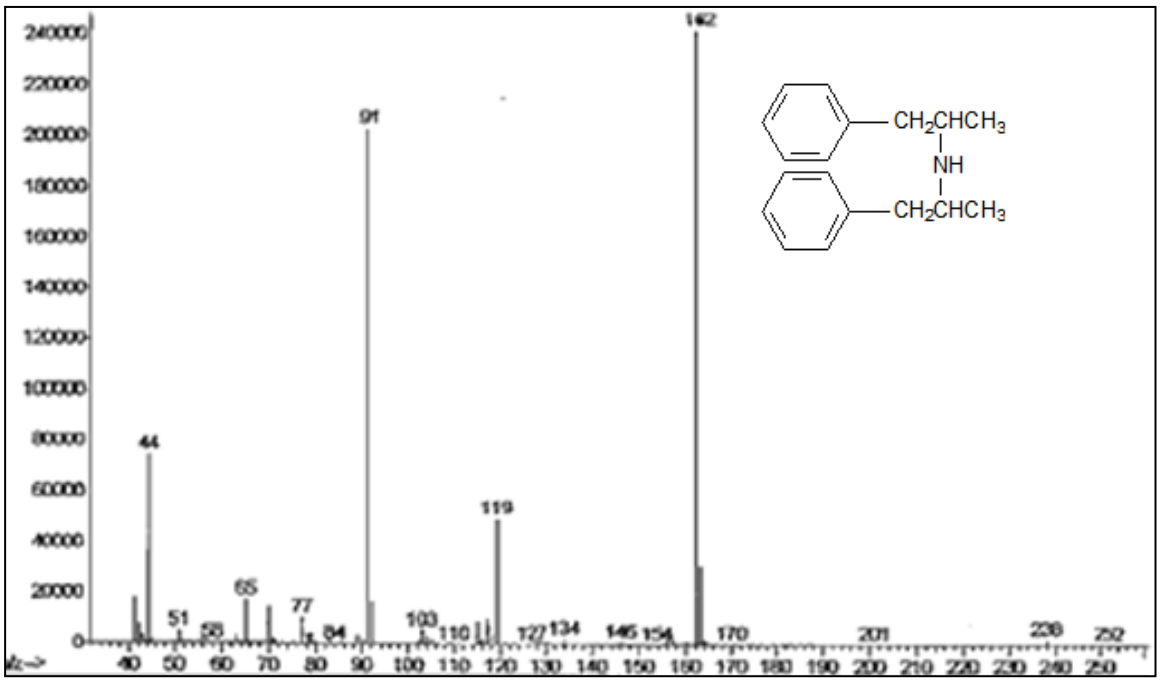


Figure 5.3 The mass spectrum of a tablet containing di(β-phenylisopropyl)amine (DPIA) showing peaks at (m/z) = 162 (base peak), 91, 44, 119, 163, 65.

increase the sensitivity of the analyses [239]. From the analyses no amphetamine was detected and only di-(β-phenylisopropyl)amine (DPIA) was detected in the tablets (Figure 5.3). Only batch one was found to contain tablets with the DPIA present.

Batches 2 and 3 gave positive results for synthetic stimulant BZP (Figure 5.4) while batches 4, 31 and 32 gave all positive results to a chlorophenylpiperazine (CCP) compound. The identification of CPP based on mass spectrometry was unable to differentiate between its three isomers 1-(2-chlorophenyl)piperazine oCPP, 1-(3-chlorophenyl)piperazine (mCPP) or 1-(4-chlorophenyl)piperazine (pCPP). Thus the three batches were analysed using ^{1}H NMR to determine the specific isomer. The

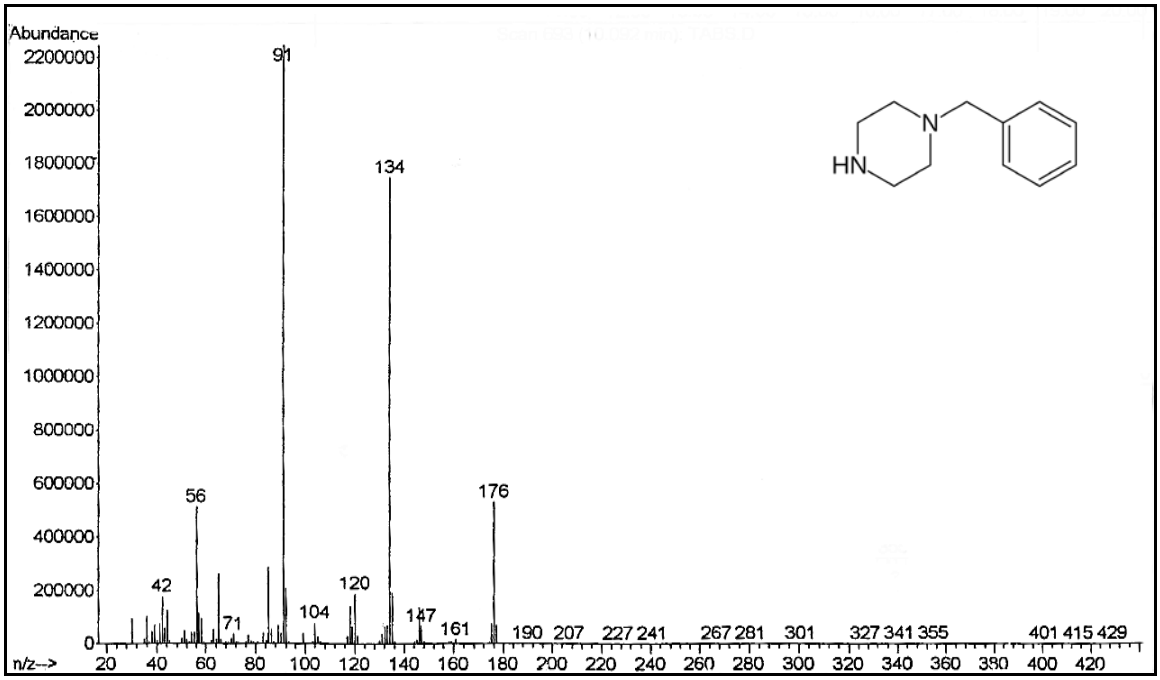


Figure 5.4 The mass spectrum of a tablet containing benzylpiperazine (BZP) showing peaks at (m/z) = 91 (base peak), 134, 56, 176, 65, 85.

analyses of the tablets were carried out by Professor Antonio Randazzo at the Faculty of Pharmacy, at the University "Federico II" of Naples.

The ^{1}H NMR spectra (Figure 5.5) do not show the exchangeable NH in MeOD given the amount of peaks in the region 3.5 – 4.0 ppm. The NMR spectrum obtained for the

aromatics excluded both the para-substitution, which is too characteristic, and the ortho-substitution, which tends to give the 4 contiguous protons coupling together, while confirming the meta-substitution, which is characteristic and could be seen on the spectrum. The aromatic region (7-8 ppm) was characterized by the presence of two doublets at 6.89 (J = 8.1 and 1.9 Hz) and 6.93 ppm (J = 8.1 and 1.9 Hz), respectively, attributable to H4' and H6' protons. The protons H4' and H6' each show the large coupling to proton H5' to give doublets, that are further split by the long range coupling to H2' proton. Furthermore, the two triplets at 7.02 (J = 1.9 Hz) and 7.23 ppm (J = 8.1 Hz), are attributed to H2', which is essentially a singlet, and H5' protons, which appears as a triplet but is actually an over laid double doublet, and their coupling to H4' and H6'

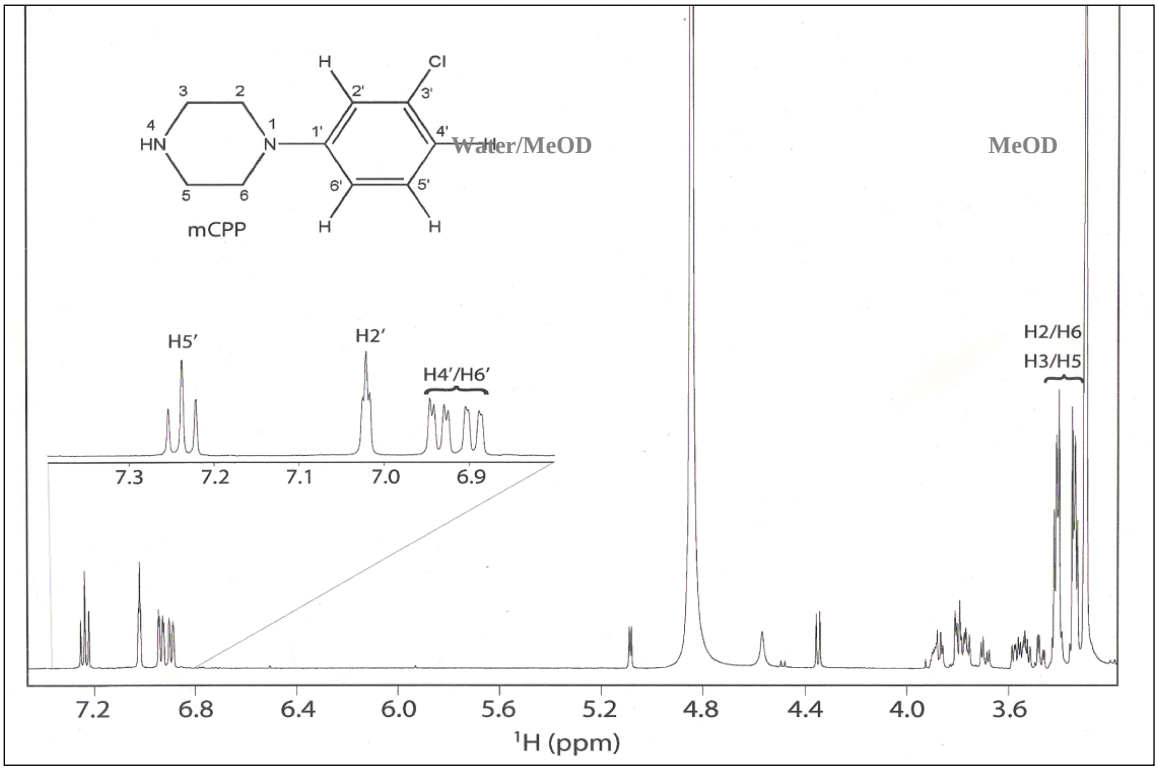


Figure 5.5 ^{1}H NMR spectrum shows the presence of 1-(3-chlorophenyl)piperazine (mCPP), as the main and only active ingredient, from an extract of a portion of ≈ 2 mg of one crushed tablet (batch 4) by 1 mL of deuterated methanol. The mCPP is characterised by the presence of two doublets, attributable to H4' and H6' protons and coupling to H5' and H2' as indicated.

protons. Hence, the ^{1}H NMR spectra confirmed the presence of mCPP molecule (Figure

5.6), which does not contain an asymmetric carbon atom and thus has no stereoisomer. Moreover, the doublets at 5.08 and 4.34 ppm, along with other signals between 3 and 4 ppm are consistent with the presence of sugars in the sample (Figure 5.5). Hence the tablets from batch 4 contained only mCPP. The presence of mCPP as the only active ingredient was also confirmed by ^{1}H NMR for batches 31 and 32.

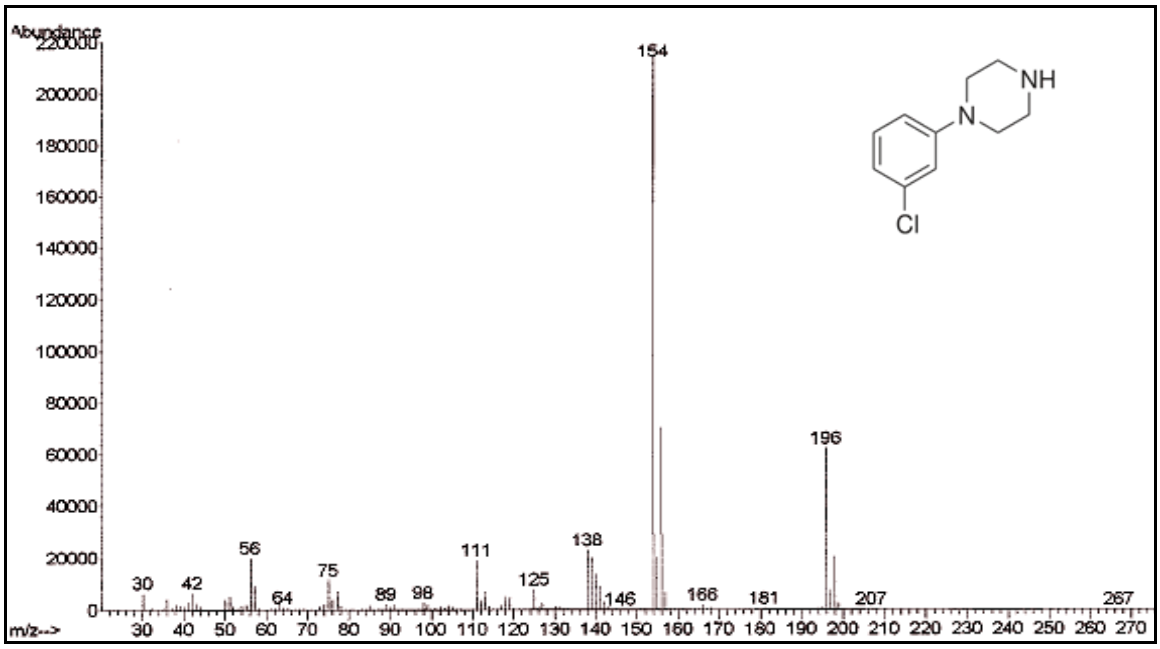


Figure 5.6 The mass spectrum of a tablet containing 1-(3-chlorophenyl)piperazine (mCPP) showing peaks at (m/z) = 154 (base peak), 196, 156, 138, 55, 111.

The tablets from batch 30 were found to contain the anabolic steroid methandrostenolone as the only active ingredient (≈ *Rt* 14.83 min, the mass spectrum of this compound had a base peak of m/z 122 with ions at m/z 121, 91, 43, 161, 147), while tablets from batches 34 - 39 were found to contain only caffeine (≈ *Rt* 10.72 min, the mass spectrum of this compound had a base peak of m/z 194 with ions at m/z 109, 67, 55, 82, 193). The tablets from batches 42 and 43 contained no psychoactive substances.

5.3.1.4 Content of psychoactive substance in 'ecstasy' tablets

Table 5.4 shows those batches that contained only MDMA with the means for the mass

and content of MDMA, quantified by UV spectrophotometry. The mean content of the batches of the MDMA tablets was 52.6 mg (SD 14.75) and batch 22 had tablets with the highest content of MDMA (mean 81.3 mg of base, SD 2.96), while batch 24 had tablets with the lowest content (mean 12.9 mg of base, SD 0.34).

Table 5.4 Batches of ecstasy tablets containing MDMA as the only active ingredient that were seized in Malta from 2006 - 2011.

Batch No.	Tablet	Mean Mass (mg)	Mean MDMA[a] (mg)	Batch No.	Tablet	Mean Mass (mg)	Mean MDMA (mg)	Batch No.	Tablet	Mean Mass (mg)	Mean MDMA (mg)
5		240 (4.65)[b]	57.8 (3.69)	15		154 (3.47)	57.7 (4.35)	25		194 (2.71)	43.5 (4.32)
6		239 (3.32)	57.1 (3.15)	16		154 (2.49)	59.0 (3.51)	26		209 (3.00)	19.6 (2.86)
7		240 (3.66)	59.3 (4.23)	17		211 (2.22)	57.1 (2.15)	27		258 (4.46)	43.8 (4.69)
8		239 (4.02)	49.8 (4.64)	18		200 (3.57)	57.2 (2.97)	28		236 (2.79)	62.8 (3.38)
9		236 (4.21)	48.8 (4.32)	19		202 (3.22)	60.6 (3.52)	29		197 (1.71)	51.5 (3.36)
10		236 (4.34)	48.4 (4.36)	20		295 (4.46)	74.1 (4.21)	33		274 (2.30)	57.5 (5.05)
11		201 (3.22)	43.6 (3.22)	21		241 (3.39)	48.8 (3.32)	40		237 (10.89)	60.2 (11.64)
12		202 (4.51)	43.6 (4.66)	22		352 (3.64)	81.3 (3.64)	41		246 (6.58)	69.3 (4.35)
13		200 (4.13)	43.2 (3.89)	23		135 (3.85)	23.6 (10.06)	44		249 (8.42)	64.3 (3.94)
14		154 (3.41)	57.4 (3.66)	24		202 (1.46)	12.9 (2.63)	45		248 (8.27)	65.3 (10.91)

[a] MDMA is the base
[b] Numbers in parentheses are the RSDs

Although in Figure 5.7 it looks like the bigger the tablet the more MDMA it contains, the correlation between the mean "MDMA content" and mean "tablet mass" was found to be low (R^2 = 0.2721) even when batches 26 and 24 were removed the correlation was still low (R^2 = 0.4104) (batches 23, 26 and 24 removed R^2 = 0.3109).

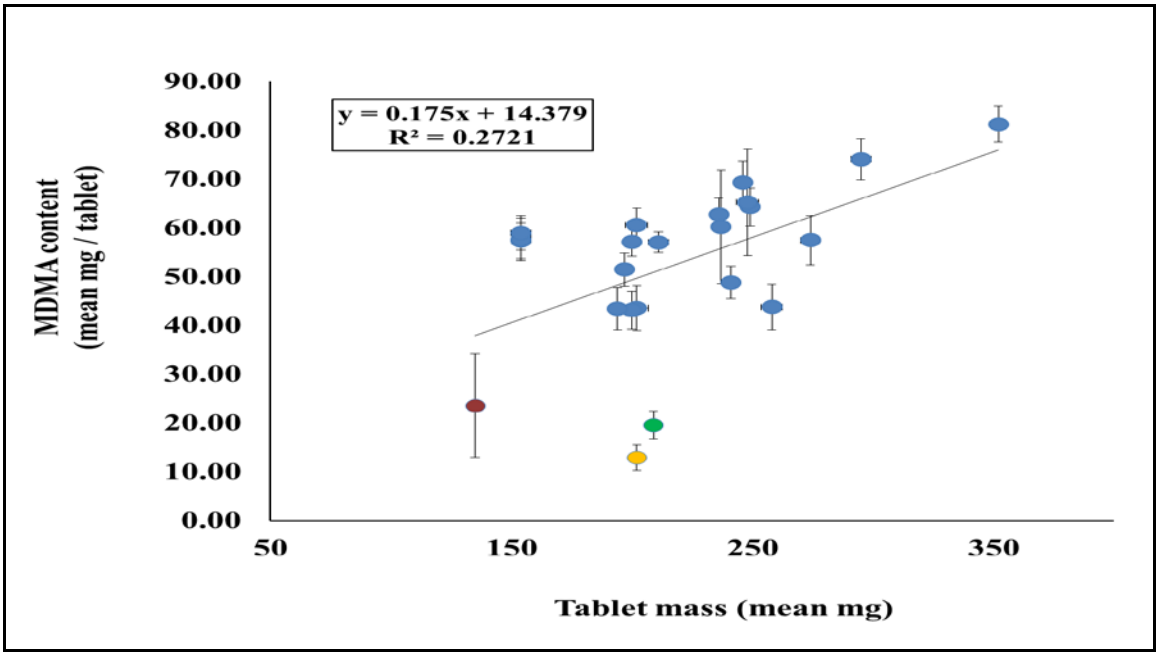


Figure 5.7 The correlation between the mean "MDMA content" and mean "tablet mass" for the batches of tablets which contained MDMA as their active ingredient (the brown spot is batch 23, the green is batch 26 and yellow batch 24).

Table 5.5 'Ecstasy' tablets containing psychoactive substances (P.S.) other than MDMA that were seized in Malta from 2006 - 2011.

Batch No.	Tablet	Mean Mass (mg)	Mean P.S. (base mg)		Batch No.	Tablet	Mean Mass (mg)	Mean P.S. (base mg)	
1		432 (3.91)[a]	DPIA[b]	/	35		177 (3.25)	caffeine	41.9 (4.08)
2		432 (4.19)	BZP	206.4 (4.08)	36		201 (3.00)		47.8 (3.26)
3		448 (3.39)		216.5 (3.31)	37		333 (1.82)		20.1 (2.00)
4		280 (4.93)	mCPP	42.2 (8.28)	38		338 (1.31)		20.0 (1.46)
30		135 (4.00)	Methandrost-enolone	10.1 (2.43)	39		364 (1.44)		22.0 (1.16)
31		206 (3.14)	mCPP	58.5 (8.51)	42		198 (1.95)	No P.S.	/
32		222 (1.45)		60.4 (2.42)	43		203 (3.88)		/
34		179 (2.24)	caffeine	42.7 (2.74)	control		177 (0.91)	bumetanide	1.0 (0.50)

[a] *Numbers in parentheses are the RSDs.*
[b] *No reference standard.*

The twelve batches of 'ecstasy' tablets which contained the active ingredients BZP (batches 2 and 3), mCPP (batches 4, 31 and 32), methandrostenolone are (batch 30) and caffeine (batches 34-39) are presented in Table 5.5. The mean content for the DPIA tablets could not be calculated because no control was available. The two batches (batches 42 and 43) of 'ecstasy' tablets were not found to contain active compounds (Table 5.5).

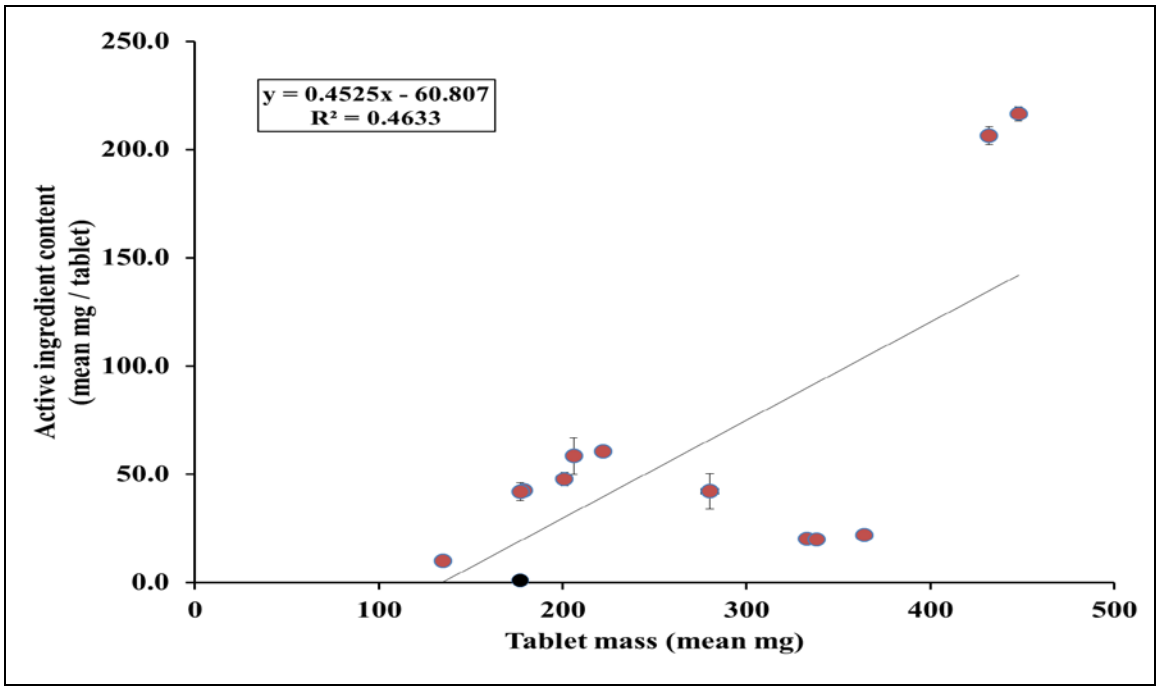


Figure 5.8 The correlation between the mean "active ingredient content" and mean "tablet mass" for the batches of tablets which contained BZP, mCPP and caffeine as their active ingredient (the black spot is the mean of control tablets bumetanide).

Similar to Figure 5.7, it seems from Figure 5.8 that the bigger the tablet the more active ingredient the tablet contains, however, the correlation between the mean "active ingredient content" and mean "tablet mass" was found to be low ($R^2 = 0.4633$).

5.3.2 Experiment B: Specific identification and enantiomeric composition of MDMA tablets

In order to investigate the isomeric content ratio a ingle tablet was selected from 22

batches known to contain MDMA. The GC-MS method described in Section 2.2.3.2 was used to determine the enantiomeric ratio. The mean for the S(+)-MDMA was 50.36% (SD 1.90%) and ranged between 46.96 – 52.95% and for the R(-)-MDMA the mean was

Table 5.6 MDMA tablets and control sample results listed as percentage (%) of S(+) and R(-)-MDMA enantiomers. The isomeric content ratios of 22 MDMA tablets were measured using software integration (Agilent Chemstation) to calculate peak area %.

Batch No.	Tablet	Logo	S(+)-MDMA %	R(-)-MDMA %	Batch No.	Tablet	Logo	S(+)-MDMA %	R(-)-MDMA %
5		omega	49.69	50.31	24		euro	51.91	48.09
8		omega	51.77	48.23	25		star	50.69	49.31
11		euro	52.04	47.96	26		smiley	52.22	47.78
14		pisces	49.49	50.51	27		question mark	52.95	47.05
17		euro	51.38	48.62	28		heart	51.98	48.02
18		D&G	49.86	50.14	29		D&G	50.76	49.24
19		heart	49.19	50.81	33		versace	51.64	48.36
20		shark	47.76	52.24	40		$E=mc^2$	52.48	47.52
21		kangaroo	49.60	50.40	41		route 66	50.31	49.69
22		letter X	46.96	53.04	44		lacoste	46.51	53.49
23		heart shaped	51.58	48.42	45		lacoste	47.19	52.81
Single MDMA enantiomers 1mg mL^{-1}								**52.09**	**47.91**
(+)-(*R,S*)-MDMA (Alltech) Control 1 mg mL^{-1}								**51.09**	**48.91**

Key: Batches 6, 7 and 9, 10 and 12, 13 and 15, 16 were from the same seizure as batches 5, 8, 11 and 14.

49.64% (SD 1.90%) and ranged between 47.05 - 53.49% (GC area peak %). The enantiomeric ratio composition (S(+)-/R(-)-) for all the tablets was ~ 1 : 1. The percentage for the S(+) and R(-) – MDMA of the analysed tablets are shown in Table 5.6.

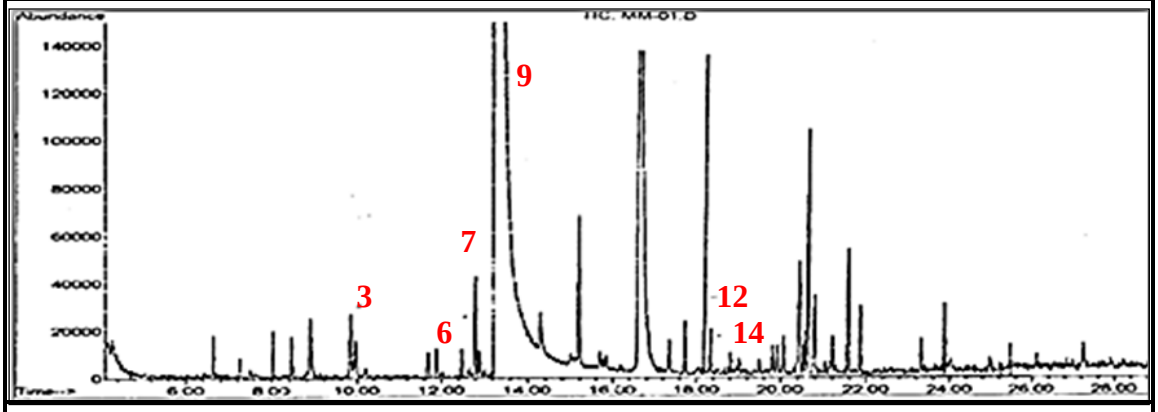
5.3.3 Experiment C: An investigation to determine the organic impurity profile of MDMA tablets

The impurity profiles of 22 MDMA tablets, from different batches as indicated in Table 5.1, were carried out using GC-MS (EI) analyses as described in Section 2.2.3.3. The chemical profiles of MDMA tablets in this study are taken to be the coextracted and manufacturing impurities, also known as impurity profiles. Each MDMA tablet has a characteristic chemical pattern of impurities which is also known as a profile. Typical total ion current chromatograms of extracted MDMA tablets are shown in Figure 5.9.

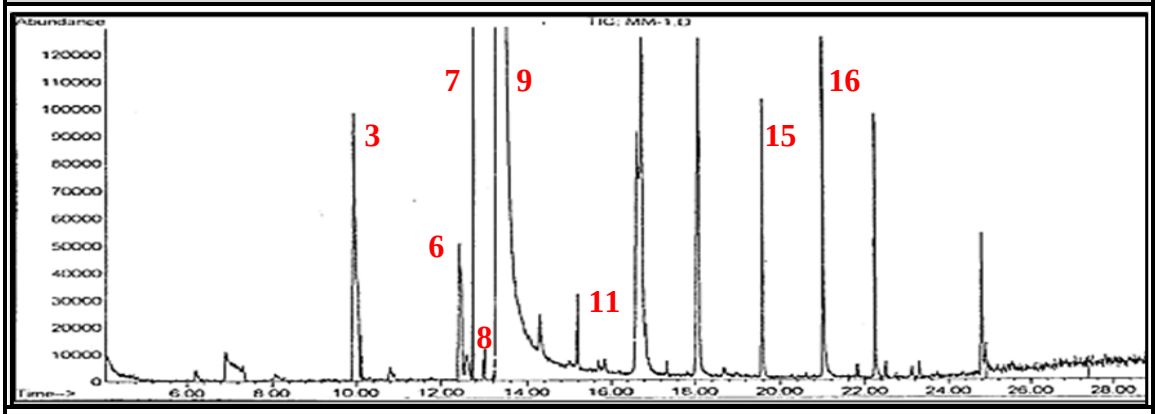
The retention times and mass spectra peaks of the MDMA (only main active ingredient) and other compounds identified in the tablets are listed in Table 5.7 below. The identification of compounds was based on the EI mass spectra published in literature or by National Institute of Standards and Technology (NIST) library PMW_TOXR.L. Thirteen different compounds that are known precursors or reaction intermediates for the MDMA synthesis were identified, these included piperonal, isosafrole, 3,4-MDP2P, DMMDA and N-formyl MDMA. Not all the peaks could be identified because their corresponding mass spectra were not recorded in the literature. In this study the GC-MS impurity profiles were treated like fingerprints to link / discriminate the tablets from the different batches seized on different occasions.

The most detected impurities in the analysed tablets were 3,4-MDP2P (86.4%, n = 19), followed by piperonal (72.7%, n = 16) and isosafrole, 3,4-MDP2-propanol and DMMDA which were all detected in 11 tablets (50%). Other identified impurities were N-Formyl-MDMA (36.4%, n = 8), MDA and 3-Methyl-6,7-methylenedioxy-3,4-dihydroisoquinolin-1(2H)-one both detected in 6 tablets (27.3%), N-Methyl-(1,2-methylenedioxy)-4-(1-ethyl-2-aminopropyl)benzene (22.7%, n = 5) and safrole (18.2%, n = 4). Three identified impurities not related to MDMA synthesis included palmitic and stearic acid and cocaine.

White tablets with heart logo – batch 28



Pink tablets with lacoste logo – batch 44



Pink tablets with lacoste logo – batch 45

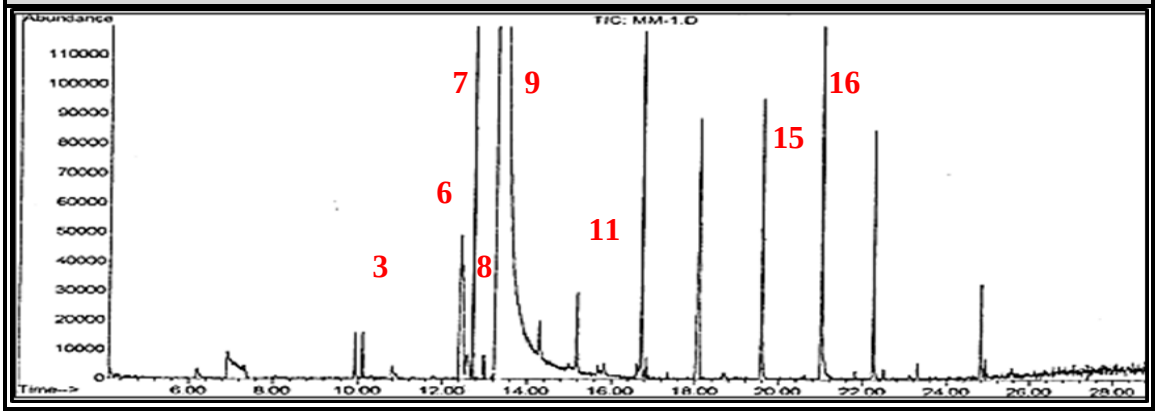


Figure 5.9 Three TIC of illicitly prepared MDMA and impurities. 3: Piperonal; 6: 3,4-MDP2P; 7: MDA (psychoactive substance); 8: 3,4-Methylenedioxy-phenyl-2-propanol; 9: MDMA; 11: 3-Methyl-6,7-methylenedioxy-3,4-dihydroisoquinolin-1(2H)-one; 12: N-Formyl MDMA, 14: N-Acetyl-MDMA, 15: Palmitic acid and 16: Stearic acid. The operating conditions were in the EI mode as described in Chapter 2, Section 2.2.3.3.

Table 5.7 The retention times (*Rts*) and the 5 major EI spectral data (first m/z is the base peak) of the impurities found in 22 MDMA tablets. The EI mass spectra of the compounds were identified from the literature [121, 272, 273] (numbers in brackets are the EI mass spectra references).

No.	Chemical Name	≈ *Rt*	Mass Spectral data – principal peaks at *m/z*
1	Safrole	8.39	162, 131, 104, 135, 103 [272]
2	3,4-(Methylenedioxy)-phenylpropane	9.41	135, 164, 77, 51, 50 [121]
3	Piperonal	10.02	149, 150, 121, 63, 65 [272]
4	Isosafrole	10.84	162, 131, 104, 103, 161 [272]
5	Piperonyl alcohol	11.58	152, 135, 93, 65, 123 [121]
6	3,4-MDP2P	12.40	135, 178, 77, 136, 79 [272]
7	MDA *	12.81	44, 135, 136, 77, 51
8	3,4-Methylenedioxy-phenyl-2-propanol (3,4-MDP2-propanol)	12.98	135, 136, 180, 77, 106 [121]
9	MDMA	13.41	58, 135, 77, 105
10	DMMDA	14.22	72, 56, 44, 73, 58 [273]
11	3-Methyl-6,7-methylenedioxy-3,4-dihydroisoquinolin-1(2H)-one	15.26	148, 190, 147, 205, 188 [272]
12	N-Formyl-MDMA	18.42	86, 162, 58, 135, 77 [272]
13	N-Methyl-(1,2-methylenedioxy)-4-(1-ethyl-2-aminopropyl)benzene	18.61	58, 162 , 77 135, 194 [121]
14	N-Acetyl-MDMA	18.91	58, 162, 100, 43, 135 [273]
15	Palmitic acid	19.08	73, 60, 57 55 129 (M^+ 256)
16	Stearic acid	21.15	73, 60, 57 55 129 (M^+ 284)
17	Cocaine *	21.56	182, 82, 83, 105, 303

**** These compounds are psychoactive substances***

Table 5.8 Impurities from precursor chemicals and reaction by-products found in MDMA tablet batches from the analysed tablets (the same numbers for the impurities used in Table 5.7 (Note: a =3-Methyl-6,7-methylenedioxy-3,4-dihydroisoquinolin-1(2H)-one and b = N-Methyl-(1,2-methylenedioxy)-4-(1-ethyl-2-aminopropyl)benzene)

Tablets Batch No.	Safrole	3,4-MDPP	Piperonal	Isosafrole	Piperonyl alcohol	3,4-MDP2P	MDA	3,4-MDP2-propanol	DMMDA	3-Me-6, 7-MD-3,4-DHI -1(2H)-one (a)	N-Formyl-MDMA	N-M-(1,2-MD)-4-(1-El-2-APB (b)	N-Acetyl-MDMA
	1	2	3	4	5	6	7	8	10	11	12	13	14
5	✓		✓	✓					✓			✓	
8	✓		✓	✓					✓			✓	
11				✓		✓			✓	✓	✓		
14	✓				✓				✓	✓	✓		
17			✓			✓	✓	✓		✓			
18			✓			✓	✓	✓	✓				
19						✓		✓	✓				
20			✓	✓		✓					✓		
21				✓		✓			✓		✓		
22			✓	✓		✓		✓	✓				
23			✓	✓		✓		✓	✓			✓	
24	✓		✓			✓		✓	✓			✓	
25			✓	✓		✓					✓		
26			✓	✓		✓					✓		
27			✓			✓	✓	✓		✓			
28			✓			✓	✓				✓		✓
29		✓		✓		✓			✓				✓
33			✓	✓		✓		✓				✓	
40		✓			✓	✓		✓					
41		✓	✓			✓					✓		
44			✓			✓	✓	✓		✓			
45			✓			✓	✓	✓		✓			

The impurities that were found to co-occur in the same chemical profile included piperonal and 3,4-MDP2P (31.8 %, n = 7); and isosafrole, piperonal and 3,4-MDP2P (27.3%, n = 6). Table 5.8 shows the impurities from precursor chemicals and reaction by-products found in MDMA from the analysed tablets. Other groups of impurities from precursor chemicals found together in the same chemical profiles included: safrole, piperonal and 3,4-MDP2P; isosafrole and 3,4-MDP2P; and safrole, isosafrole and piperonal. Moreover, the impurity from the precursor chemical 3,4-MDP2P was found alone in only one chemical profile. The key intermediate seemed to be 3,4-MDP2P for all the chemically profiled MDMA tablets. The compound 3,4-MDP2P was detected in most of the (86.4%, n = 22) tablets.

5.3.4 Experiment D: Determination of major excipients of 'ecstasy' tablets

A single tablet was used from each of the 35 batches (batches 4-18, 21-23, 25, 26, 29, 31-41, 44 and 45, 35 out of the 45 batches were used as tablets from the other batches were all used) to determine major excipients. The detected excipients were (% number of tablets) were lactose (34.3%, n = 12, Figure 5.10), sorbitol (25.7%, n = 9), dibasic calcium phosphate (DCP) (22.9%, n = 8) and starch (17.1%, n = 6) (Table 5.9). Nearly all of the tablets contained the lubricant magnesium stearate (94.1%, n = 32).

Table 5.9 Characteristic FTIR spectral peaks of reference substances that are used as major excipients in tablets (scan range 4000 cm^{-1} - 500 cm^{-1}).

Major Excipients	FTIR – Transmittance – peaks (wavenumber, cm^{-1})				
Lactose	3522	3381	2900	1657	1427
Sorbitol	3390	2933	2105	1632	1414
Starch	3536 - 3215	2926	1644	1142	1005
DCP	3556	~3000	2407	1667	1250

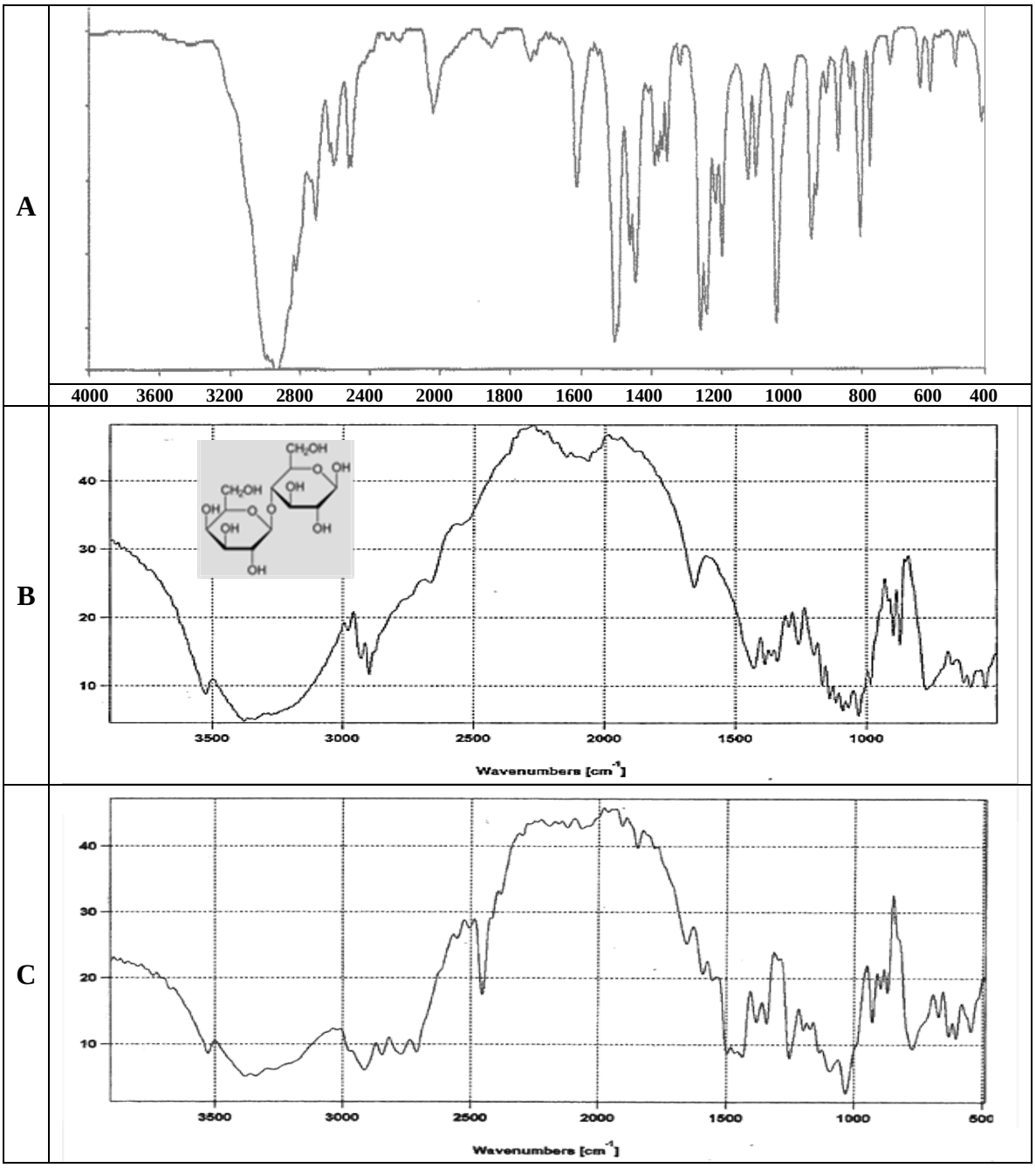


Figure 5.10 FTIR transmittance spectra of: (A) a standard MDMA-HCl [212] (peaks at 2710 cm^{-1} correspond to the N-H stretching of amine salt, that at 2458 cm^{-1} to the C-H stretching of CH_3 group, that at 1488 cm^{-1} to the C-H band, those at 1244 and 1030 cm^{-1} to the C-O-C stretching of methoxy group and the 930 cm^{-1} to the C-O stretching), (B) a sample of lactose (peaks from 3600 to 3200 cm^{-1} correspond to the C-O-H stretching of lactose alchol groups and the peaks standing within 1049 to 1160 cm^{-1} correspond the C-O-C stretching of ether unit bonds), (C) an 'ecstasy' tablet containing MDMA-HCl and lactose (batch 18, additional lactose peaks at 1140 cm^{-1}, 1114 cm^{-1}, 1070 cm^{-1} and 1018 cm^{-1}).

Table 5.10 The detected major excipients in MDMA tablets.

Batch No.	Tablet	Logo	Excipient	Batch No.	Tablet	Logo	Excipient
5-7		omega	sorbitol	25		star	lactose
8-10		omega	sorbitol	26		smiley	lactose
11-13		euro	lactose	28		heart	starch
14-16		pisces	sorbitol	29		D&G	lactose
17		euro	lactose	33		versace	lactose
18		D&G	lactose	40		E=mc^2	starch
21		kangaroo	DCP	41		Route 66	starch
22		letter X	starch	44		lacoste	lactose
23		heart shaped	starch	45		lacoste	lactose

Table 5.11 The detected major excipients in 'ecstasy' tablets that contained mCPP and caffeine as their active ingredient.

Batch No.	Tablet	Logo	Active Ingredient	Excipient
4		lacoste	mCPP	lactose
31		tulip	mCPP	DCP
32		mercedes	mCPP	DCP
34		question mark	caffeine	DCP
35		question mark	caffeine	DCP
36		heart	caffeine	starch
37		heart	caffeine	DCP
38		rolex crown	caffeine	DCP
39		rolex crown	caffeine	DCP

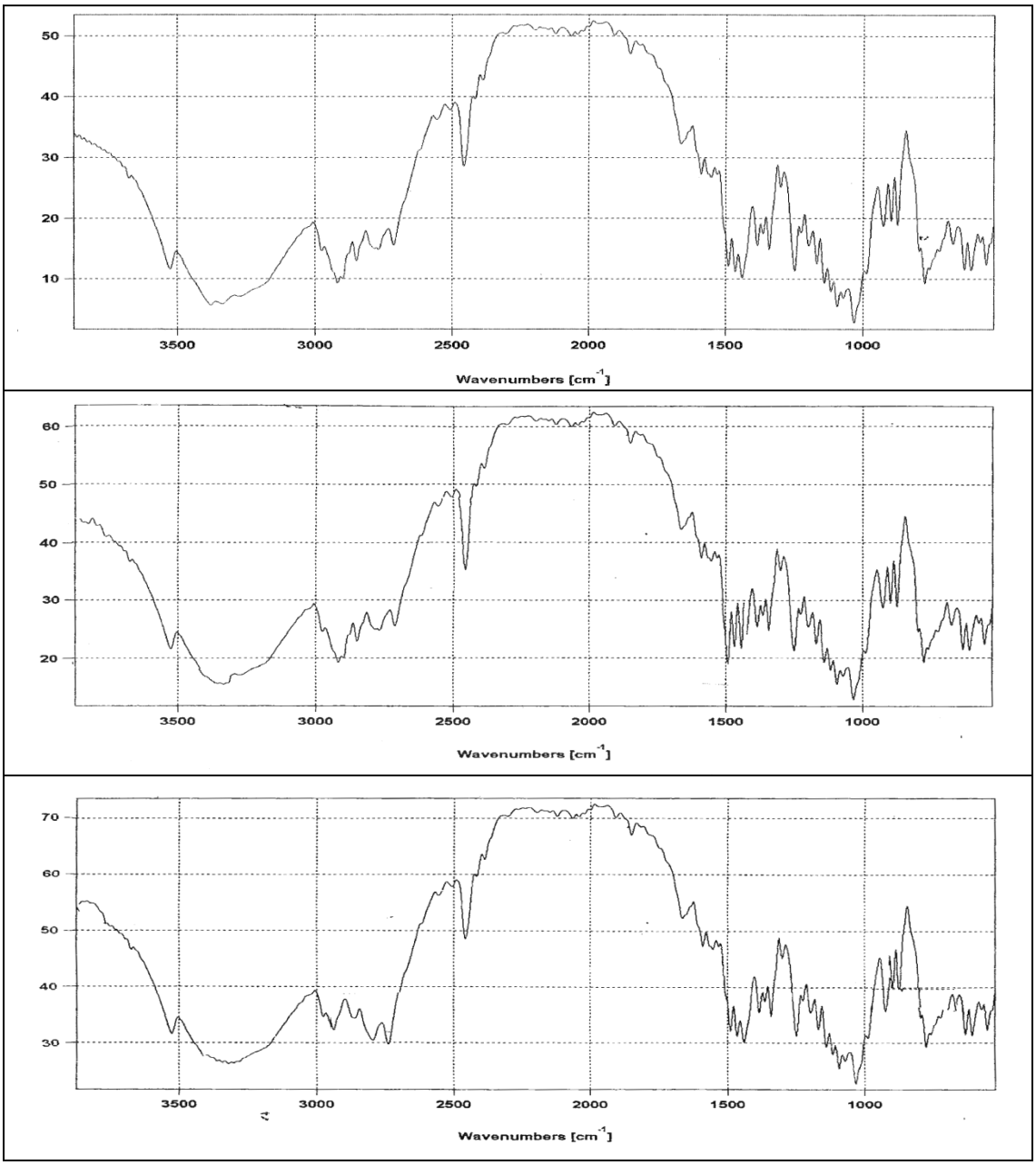


Figure 5.11 FTIR transmittance spectra of three tablets, white with euro logo, taken from three different batches (batches 11-13).

Less than half of the MDMA tablets were found to contain lactose as the main excipient (42.3 %, n = 11), followed by sorbitol (34.6%, n = 9), starch (19.2%, n = 5) and one tablet containing DCP as the major excipient (Table 5.10). The detected major excipients in the 'ecstasy' tablets containing the active ingredients mCPP and caffeine were DCP, lactose and starch. The predominant excipient detected in the 'ecstasy' tablets containing

the active ingredients mCPP and caffeine was DCP (7 tablets out of 9), while the excipients lactose and starch were detected in one tablet each (Table 5.11). The presence of both DCP and magnesium stearate were confirmed by SEM/EDX analyses.

The FTIR transmittance spectra of the MDMA-only tablets were fairly similar but differed in the fingerprint region (1500 – 900 cm^{-1}). Tablets with similar physical features (shape, colour and logo) had very similar FTIR transmittance spectra (Figure 5.11).

5.3.5 Experiment E: Elemental profiling of 'ecstasy' tablets

A total of 37 'ecstasy' tablets from batches 2, 4-18, 21, 23, 24, 26-41, 44 and 45 were analysed (37 not 45 batches were used as tablets from the other batches were all used). Almost three quarters of the tablets (70.3%, n = 26) contained MDMA as the only active ingredient. The other tablets contained other active ingredients which included BZP batch 2, mCPP batches 4, 31 and 32, an anabolic steroid, batch 30, and caffeine, batches 34-39. The tablets used for analyses were each mounted on a SEM sample stub using double-sided carbon adhesive tape. The tablets were then coated with a thin carbon layer and then each analysed by SEM/EDX (Section 2.2.3.5). A total of ten ions were detected in the analysed tablets, which were: sodium (Na), magnesium (Mg), aluminium (Al), silicone (Si), phosphorus (P), sulphur (S), chlorine (Cl), potassium (K), calcium (Ca) and titanium (Ti). Because the sensitivity of energy dispersive X-ray analysis is relatively low ($\geq$ 0.1%), trace elements could not be detected.

The chloride ion was the most commonly detected ion (97.3%, n = 36) followed by the magnesium ion (91.9%, n = 34) and the silicon ion (83.8%, n = 31). The presence of the detected elemental ions was mainly due to the excipients used in the analysed 'ecstasy' tablets. More than half (51.4%, n = 19) of the analysed tablets could be differentiated by the detected elemental ions. The most common group of ions found together with no other ions on the tablets, containing MDMA as the only active ingredient, were the Mg, Si and Cl, ions which were found on 21.6% (n = 8) of the tablets (Table 5.12).

Table 5.12 Percentage weights of elemental ions Mg, Si and Cl detected on the surface of MDMA tablets by SEM/EDX analyses.

Batch No.	Tablet	Logo	MDMA Tablets Characterised by their Elemental Ions - Weight %		
			Mg	Si	Cl
11			19.65	17.37	62.98
12		euro	22.65	18.13	59.22
13			17.37	25.24	57.39
17		euro	24.50	34.01	41.48
26		smiley	23.52	32.57	43.91
27		question mark	29.05	46.21	24.74
28		heart	23.90	31.16	44.94
33		versace	19.35	21.87	58.78

Six MDMA tablets with a mixture of colour and logos but which all contained the same ions, which were Mg, Si and Cl, were investigated for difference in % weight (Table 5.12) of the detected elements in their profile (Figure 5.12). The inorganic profile for tablet from batch 11, white tablet with euro logo (tablet 1 in Figure 5.12), was very similar to the profile of tablet from batch 33, white tablet with versace logo (tablet 6 in

Figure 5.12). The inorganic profiles of tablets 2, 3 and 5 shown in Figure 5.12 were very similar. The tablets with reference to Figure 5.12 were: tablet 2, white with euro logo from batch 17, tablet 3, pink with smiley logo from batch 26, and tablet 5, white with heart logo from batch 28. The elemental profile of tablet 4, blue with question mark logo from batch 27, in Figure 5.12, was different from the rest of the other tablets.

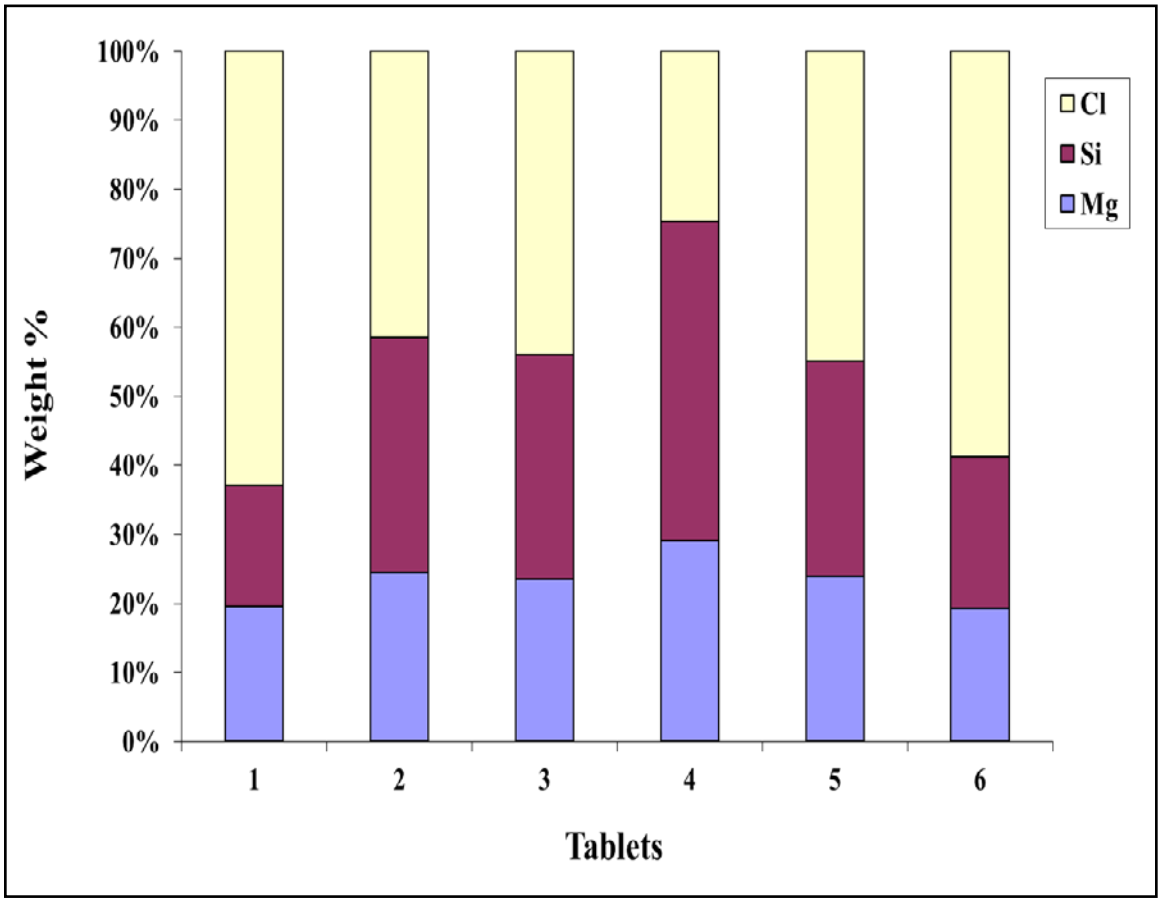


Figure 5.12 Similar inorganic profiles (Weight % of the elemental ions Mg, Si and Cl) of six MDMA tablets, shown in Table 5.12, with a mixture of colour and logos seized on different occasions.

From surface analyses conducted on MDMA tablets from batches 5-10, 14-16, 23, 29, 40, and 41 (Table 5.13) six elemental ions were detected which included Na, Mg, Al, Si, S and Cl. The elemental ion Cl was detected on all the tablets, while Mg was on 12 tablets (Table 5.13). Moreover, from the surface analyses of another group of MDMA tablets

Table 5.13 Percentage weights of the elemental ions Na, Mg, Al, Si P, S and Cl detected on the surface of MDMA tablets by SEM/EDX analyses.

Batch No.	Tablet	Logo	MDMA Tablets Characterised by their Elemental Ions - Weight %					
			Na	Mg	Al	Si	S	Cl
5			3.47	10.32	3.57	21.24	3.62	57.78
6		omega	4.32	7.53	4.68	22.47	4.76	56.24
7			1.24	11.15	2.74	24.82	3.13	56.92
8			1.37	23.06	/	28.27	4.75	42.55
9		omega	2.24	20.48	0.84	29.37	3.80	43.27
10			1.90	21.45	1.03	27.22	4.13	44.27
14			1.84	3.64	/	2.55	/	91.98
15		pisces	2.83	3.89	/	3.37	/	89.91
16			4.33	1.28	/	5.23	/	89.16
23		heart shaped	7.79	12.44	3.68	18.61	1.96	55.51
29		D&G	/	21.01	6.95	21.06	/	50.98
40		$E = mc^2$	/	/	/	/	0.23	99.77
41		route 66	/	0.49	0.99	/	/	98.52

from batches 18, 21, 24, 44 and 45 seven elemental ions were detected which were Na, Mg, Al, Si, P, Cl, and Ca (Table 5.14). The elemental ions Mg, Si, Cl and Ca were detected on all five tablets (Table 5.14).

Table 5.14 Percentage weights of elemental ions Na, Mg, Al, Si P, S, Cl and Ca detected on the surface of MDMA tablets by SEM/EDX analyses.

Batch No.	Tablet	Logo	MDMA Tablets Characterised by their Elemental Ions (Weight %)						
			Na	Mg	Al	Si	P	Cl	Ca
18		D&G	3.23	21.78	2.53	26.05	/	44.52	1.89
21		kangaroo	/	17.55	/	16.49	22.80	25.02	18.14
24		letter X triangular	/	16.71	/	23.61	/	58.06	1.08
44		lacoste	/	18.75	/	25.92	/	51.12	4.21
45			/	19.36	/	22.32	/	55.54	2.78

A comparison of tablets with different active ingredients

Surface analyses were conducted on five tablets containing BZP, mCPP and an anabolic steroid (batches 2, 4, 30-32) seven elemental ions were detected which were Na, Mg, Al, Si, P, Cl and Ca (Table 5.15). The elemental ions Cl was detected on four tablets, Mg on three tablets and the ions P and Ca were detected on two tablets respectively (Table 5.15). No elemental ions were detected on the anabolic steroid tablet (batch 30) (Table 5.15).

Table 5.15 Percentage weights of elemental ions Na, Mg, Al, Si P, S, Cl and Ca detected on the surface of 'ecstasy' tablets (BZP, mCPP and anabolic steroid) by SEM/EDX analyses.

Batch No.	Tablet	Logo	Active Ingredient	'Ecstasy' Tablets Characterised by their Elemental Ions (Weight %)						
				Na	Mg	Al	Si	P	Cl	Ca
2		diamond shaped	BZP	/	1.41	/	0.84	/	95.86	/
4		crocodile (lacoste)	mCPP	/	12.48	/	/	/	87.52	/
30		heart shaped	anabolic steroid	no ions						
31		tulip	mCPP	1.43	/	0.43	/	28.93	29.49	39.72
32		mercedes	mCPP	/	2.59	/	/	37.04	21.25	39.12

From the surface analyses carried out on six tablets from batches 34-39, containing caffeine as the only active ingredient, ten elemental ions were detected which were Na, Mg, Al, Si, P, S, Cl, K, Ca and Ti (Table 5.16). While the Mg, Si, P and Cl ions were detected on the surface of all six tablets the Al and Ca ions were detected on five tablets (Table 5.16).

The weight % of the detected 10 elemental ions together with the active ingredient of the tablets was used as variable in a multivariate analysis to link (similarity in the number of

Table 5.16 Percentage weights of elemental ions Na, Mg, Al, Si P, S, Cl, K, Ca and Ti detected on the surface of 'ecstasy' tablets, containing caffeine as the only active ingredient (tablets from batches 34-39), by SEM/EDX analyses.

Batch No.	Tablet	Logo	'Ecstasy' Tablets Characterised by their Elemental Ions (Weight %)									
			Na	Mg	Al	Si	P	S	Cl	K	Ca	Ti
34		question mark	/	16.40	3.70	25.64	20.67	2.59	13.34	/	14.98	2.59
35			/	12.67	/	15.87	29.95	/	7.12	/	25.85	8.65
36		heart	7.33	39.75	4.61	7.67	5.51	19.26	15.87	/	/	/
37			/	22.26	3.45	39.26	14.29	1.35	8.70	/	8.77	1.92
38		rolex crown	/	11.14	4.18	5.03	29.11	/	10.55	/	26.80	13.09
39			/	38.84	3.73	10.36	8.99	13.42	13.14	3.06	8.46	/

attributes of tablets from different batches) or differentiate between the tablets from the batches. The data matrix obtained was examined by hierarchical cluster analysis using a dendrogram (Figure 5.13). The dendrogram (cut-off < 2) shows the following linkage: a) tablets from batches 5-7, b) tablets from batches 8-10, c) tablets from batches 11-13, 24, 33, 44 and 45, d) tablets from batches 14-16, e) tablets from batches 17, 26 and 28 and f) tablets from batches 40 and 41. The tablets from the other batches (2, 4, 18, 21, 23, 27, 29-32, 34-39) were differentiated (Figure 5.13).

5.3.6 Linkage between batches

From chemical characterisation it transpired that there was a high possibility of linkage

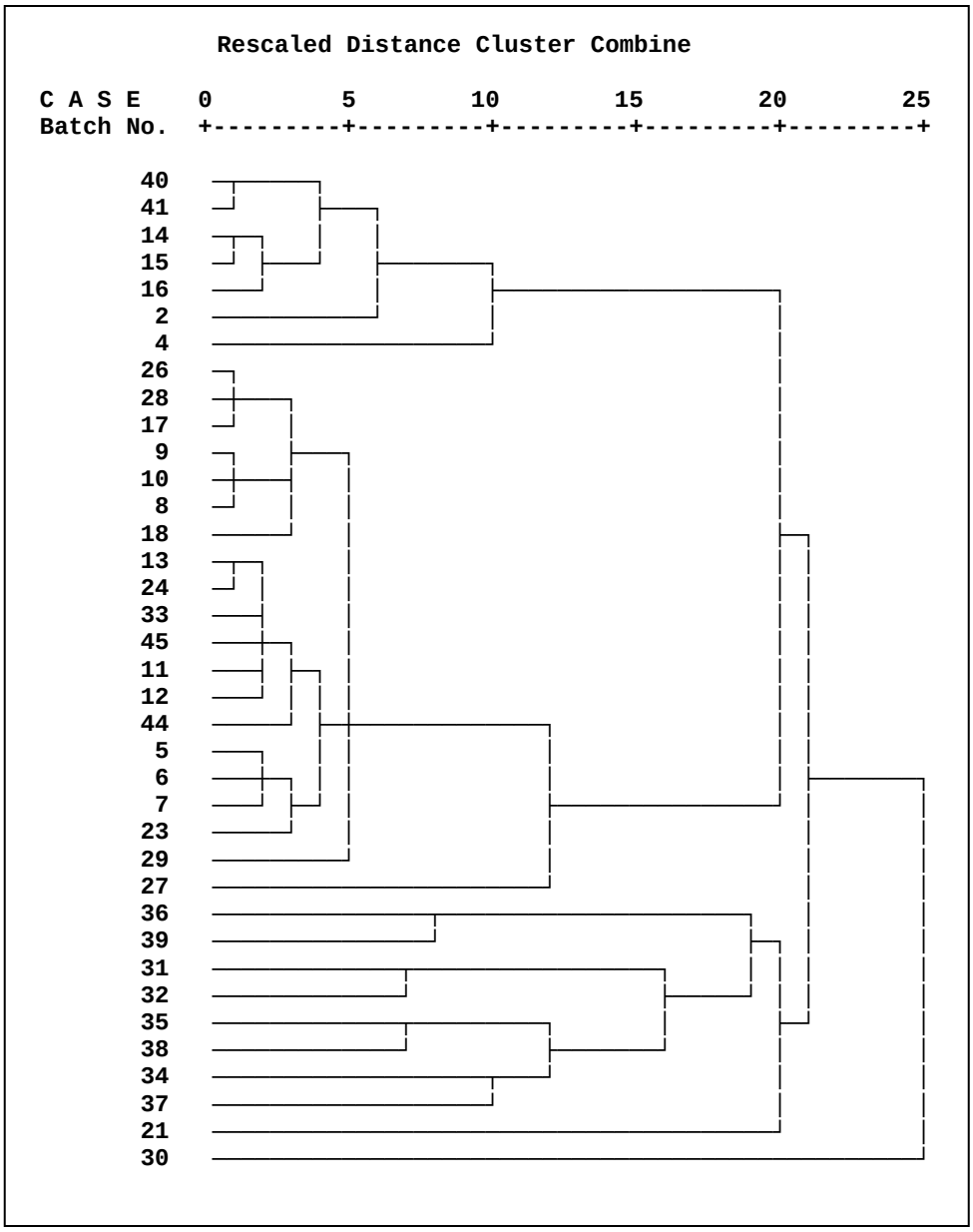


Figure 5.13 Dendrogram resulting from hierarchical cluster analysis of 'ecstasy' tablets for weight % of the detected elemental ions and active ingredients.

between batches 5 to 7, blue tablets with omega logo, batches 8 to 10, green tablets with omega logo, batches 11 to 13, white tablets with euro logo, batches 14 to 16, orange tablets with pisces logo and batches 44 and 45 pink tablets with lacoste logo (Table 5.17). The batches from 5 to 16 were all from the same seizure while batches 44 and 45 were from different seizures.

Table 5.17 Batches of 'ecstasy' tablets with similar mass and chemical characteristics indicative of a possible linkage between the respective batches (batches 5 – 16 from same seizure and batches 44 and 45 from two different seizures).

Batch No.	Tablet	Logo	Mass (mg)	Main Active Ingredient (mg)	Enantiomer Ratio S(+) / R(-)	Organic Impurity Profile	Major Excipient	Elemental Profile - % Weight						
								Na	Mg	Al	Si	S	Cl	Ca
5		omega	240 (4.65)	57.8 (3.69)	49.69 / 50.13	profiles similar (tablets from batches 5 and 8, Table 5.8)	sorbitol	3.47	10.32	3.57	21.24	3.62	57.78	/
6			239 (3.32)	57.1 (3.15)				4.32	7.53	4.68	22.47	4.76	56.24	/
7			240 (3.66)	59.3 (4.23)				1.24	11.15	2.74	24.82	3.13	56.92	/
8		omega	239 (4.02)	49.8 (4.64)	51.77 / 48.23		sorbitol	1.37	23.56	/	28.27	4.75	42.55	/
9			236 (4.21)	48.8 (4.32)				2.24	20.48	0.84	29.37	3.80	43.27	/
10			236 (4.34)	48.4 (4.36)				1.90	21.45	1.03	27.22	4.13	44.27	/
11		euro	201 (3.22)	43.6 (3.22)	52.04 / 47.96	Profiles for tablets from batches 11 and 14, Table 5.8	lactose	/	19.65	/	17.37	/	62.98	/
12			202 (4.51)	43.6 (4.66)				/	22.65	/	18.13	/	59.22	/
13			200 (4.13)	43.2 (3.89)				/	17.37	/	25.24	/	57.39	/
14		pisces	154 (3.41)	57.4 (3.66)	49.49 / 50.51		sorbitol	1.84	3.64	/	2.55	/	91.98	/
15			154 (3.47)	57.7 (4.35)				2.83	3.89	/	3.37	/	89.91	/
16			154 (2.49)	59.0 (3.51)				4.33	1.28	/	5.23	/	89.16	/
44		lacoste	249 (8.42)	64.3 (3.94)	46.51 / 53.49	profiles similar (Table 5.8)	sorbitol	/	18.75	/	25.92	/	51.12	4.21
45			248 (8.27)	65.3 (10.9)	47.19 / 52.81			/	19.36	/	22.32	/	55.54	2.78

Key: The numbers in columns mass and main active ingredient are the means and the numbers in parenthesis are the RSD

5.4 Discussion

This part of the study, which focused on the chemical characterisation of batches of tablets seized in Malta over a five year period from 2006 - 2011, showed that most of the seized batches were 'ecstasy' tablets containing a racemic mixture of MDMA. The organic impurity profiling of MDMA tablets using GC-MS analyses helped detect impurities which served to infer two plausible synthetic pathways which included the Leuckart and reductive amination reactions, while the TIC profiles were used as fingerprints to differentiate or link batches of MDMA tablets seized on different occasions. The major excipients most commonly used in the seized batches, which were lactose and sorbitol, were also determined using FTIR transmittance spectroscopy. Elemental profiling of 'ecstasy' tablets from the different batches using SEM/EDX, which was used to further link or differentiate between the batches, also helped determine other excipients used in the production of 'ecstasy' tablets. The conjoint use of organic and elemental profiling, which provided a more definite profile of the analysed 'ecstasy' tablets, demonstrated their potential for use in a forensic 'ecstasy' tablets intelligence perspective. Using the above methods it was possible to link a total of 14 batches of 'ecstasy' tablets from three seizures and differentiate between the other batches.

In ***experiment (A)*** 'ecstasy' tablets were analysed to determine and quantisise the psychoactive substances if present. The majority of the 'ecstasy' tablets seized in Malta over the 5 year period (2006 - 2011) were MDMA-only tablets (n = 30 batches, 66.7%). However, 'ecstasy' tablets containing other psychoactive substances, as the only active ingredient, such as caffeine, mCPP, BZP and DPIA (n = 12 batches, n = 26.7%), or no active ingredients (2 batches) or unrelated chemicals such as the anabolic steroid methandrostenolone, found in one batch of tablets, were also seized during the study period. The finding of 'ecstasy' tablets containing other psychoactive substances instead of MDMA, especially during the shortage of MDMA, was also observed in Europe [205].

The mean content of the batches of MDMA-only tablets was 52.6 mg (SD 14.75, range 12.9 – 81.3 mg), which was fairly similar to that reported in England and Wales which ranged between 33.1 mg and 71.0 mg (base) / tablet [39, 275], for the same period, 2006 - 2011. The range of the mean content of MDMA in the analysed tablets fell nearly in the centre of the range for the mean content of MDMA in ecstasy tablets seized in Europe which ranged between 3 and 113 mg (base) / tablet during the period of study (56, 311-313]. The mean MDMA (base) content of tablets (weight to weight) was 19.5%, which was determined from a sample from a population of 33,158 MDMA-only tablets obtained in 19 seizures (30.96% of all seized 'ecstasy' tablets, 107,098 tablets, in Malta during the period of study) during the years 2006 - 2011. According to the EMCDDA 2010 Annual Report "there were no clear trends in the MDMA content" in the tablets found in Europe [54], thus batches of MDMA tablets in Europe had different dosage as was determined in this study.

Six batches (batches 34-39) of round shaped tablets coloured blue and green with question mark, heart and rolex crown logos were found to contain the psychoactive compound caffeine only. It is not uncommon to find 'ecstasy' tablets containing caffeine as an adulterant or as the only psychostimulant substance [276]. The mean purity of these tablets for the compound caffeine was 32.42 mg (base) / tablet. Although caffeine in low doses is less harmful than MDMA [276] repeat users of 'ecstasy' tablets with high doses of caffeine may be putting themselves at risk for health problems. While low or intermediate doses of caffeine (< 100 – 250 mg / day) have a positive effect, such as increased mental performance and alertness, high doses of caffeine (300 - 800 mg / day) could cause serious health problems such as substance-induced anxiety, nervousness and insomnia mood [277]. Thus the use of ≥ 8 tablets of tablets from batches 34-36 or ≥ 15 tablets from batches 37-39 could have caused serious health problems. A caffeine tablet can promote acute toxicity if taken with 'ecstasy' containing MDMA, which is a serious drug interaction [278]. Although it is not known why this happens, it was suggested that caffeine might blocks the adenosine A_2 receptors and by so doing potentiate the DA release, thus increasing MDMA-related hyperlocomotion [279].

Two other batches of tablets which were seized in Malta were found to contain the psychoactive compound BZP (batches 2 and 3) had a mean dosage of 211.5 mg (base) / tablet. These two batches consisted of diamond and round shaped white tablets with no logos. The substance BZP, which can be synthesised from piperazine monohydrochloride and benzyl chloride [280], is a central nervous system stimulant and has about 10% relative potency that of amphetamine [281]. Little is known about the toxicity of BZP, however in New Zealand where this substance was used as a recreational drug since 2000 there were some incidents which resulted in medical emergencies [280]

Three other batches of 'ecstasy' tablets which were seized in Malta were found to contain the psychoactive substance mCPP (batches, 4, 31 and 32) at a mean dosage of 53.7 mg (base) / tablet. These three batches consisted of round shaped white tablets with lacoste, tulip and mercedes logos. The compound mCPP can be synthesised by many routes but it is commonly manufactured by diethanolamine with m-chloroaniline [282]. The compound mCPP is ingested by clubbers for its stimulant-like effects which to some extent are similar to those MDMA [283]. Although little is known about the toxicity of mCPP no fatal cases involving this ubstance has been reported in Europe [282].

Another batch of round shaped tablets, blue coloured with thumbs-up logo (batch 1), which was analysed in this study, was found to contain DPIA. This compound was not found in any of the analysed batches of tablets. The compound DPIA has two chiral centers, and therefore both a diastereomeric pair and a meso stereoisomer are usually present in illicit amphetamines prepared via the Leuckart method [284]. Illicit amphetamine samples frequently contain this substance as the main impurity which is formed during the Leuckart synthesis [285] or as a reaction intermediate following reductive amination of phenylacetone [286] but in lesser amounts than when produced during the Leuckart synthesis (Andersson K, 2008, personal communication, Scientist, Swedish National Laboratory of Forensic Science, email, 23 April). The Leuckart synthesis is known to produce a higher production of DPIA than the reductive amination process (Andersson K, 2008, personal communication, Scientist, Swedish National Laboratory of Forensic Science, email, 14 March).

The possibility that amphetamine, not DPIA, was the intended constituent cannot be excluded because the producers may not have realized that their synthesis produced DPIA and not amphetamine. Thus it could be speculated that the presence of the DPIA molecule as the main active ingredient in the tablets could indicate incomplete synthesis of amphetamine via the Leuckart route where the formamide – formic acid mixture has been used [287]. The tablets would not have provided the clubbers with the effects of amphetamine because DPIA is less potent [288].

One batch of tablets, blue coloured and heart shaped (batch 30), were found to contain the anabolic steroid methandrostenolone, was seized by the Maltese police during an EDM party. These were being sold as 'ecstasy' tablets on the basis of their appearance. The phenomenon of selling 'fake' 'ecstasy' is not new and has been reported in the literature [289]. Two other batches (batches 42 and 43) which were analysed in this study, consisted of blue coloured and round shaped tablets with euro and armani logos, very similar to 'ecstasy' tablets. These two batches, which were seized in 2011, contained tablets with excipients only and no active ingredients.

In this study no correlation was found between the means of the tablets mass and the means of the psychoactive substances contents of the analysed tablets. However, when the analysed 'ecstasy' tablets were evaluated for the uniformity of content of the active ingredient using as bench mark the European Pharmacopeia criterion 42 batches (95.5%) were found to have a uniform active ingredient in the tablets (tolerance of $\pm$ 15%) [221], indicating that most of the tablets in the batches were well made

Overall it was found that most of the 'ecstasy' tablets seized in Malta contained MDMA as the only active ingredient but other 'ecstasy' tablets containing other psychocative substances, such as caffeine, mCPP, BZP and DPIA were also found. Thus the ***hypothesis (2)*** which stated that:

> ***Seized 'ecstasy' tablets will contain MDMA as the predominant psychoactive substance.***

was not verified.

In ***experiment (B)*** the separation of MDMA enantiomers from 'ecstasy' tablets was important to determine if illegal chemists were producing chiral-specific MDMA tablets. In this experiment an achiral derivatising agent (TFAA) together with a chiral capillary column were used to separate the enantiomers. As far as is known this is a novel approach to determine the enantiomeric composition of MDMA. The results from the analysed single MDMA-only tablets from each of the 22 batches, using paired t-test, demonstrated that there was no difference in the percentage of S(+)-MDMA (M = 50.36, SD = 1.90) when compared to R(-)-MDMA (M = 49.64, SD = 1.90), t(21) = 0.895, p = 0.381, isomers in the tablets. This indicated that the MDMA present in 'ecstasy' tablets seized in Malta between the years 2006 – 2011 were racemates. The results obtained in this study were similar to those obtained by the CHAMP group where MDMA samples were also found to be racemates [179]. Hence the ***hypothesis (3)*** that:

The enantiomeric ratio of MDMA tablets will be 50:50 in illicit tablets.

was verified.

The two MDMA enantiomers have different pharmacological effects. The S(+)-MDMA, which has a stimulant effect [290], inhibit serotonin (SERT) and DA transporters, while the R(-) MDMA, which has a "hallucinogen-like" effect [290], is selective for 5-HT(2A) [291]. Moreover, while the carbon responsible for the MDMA chirality is maintained during the drug metabolism, the more active S(+)-MDMA enantiomer is metabolized faster than the R(-)-MDMA enantiomer [88, 292]. The subjective net effect of the racemic MDMA as reported by recreational users includes euphoria, emotional openness and empathy, among other [27].

The two isomers of MDMA could be separately synthesised for their different psychoactive effects. One such method described in the literature involves the ring opening of enantiopure aziridines and derivatisation from S(+)- and R(-)- alanine,

respectively, by the Grignard reagent, by methylation and detosylation to produce the S(+)- and R(-)-MDMA, which can then be converted to their hydrochlorides [291]. However, the illicit synthesis of the single MDMA enantiomers does not seem to happen and that clandestine chemists prefer to produce racemic MDMA using Leuckart reaction or reductive amination.

In ***experiment (C)*** 22 MDMA-only tablets from different batches (5, 8, 11, 14, 17-29, 33, 40, 41, 44-45) were analysed to determine some of the impurities from basic extracts after analyses by GC-MS. It has been reported that 'ecstasy' tablets from different seizures containing MDMA as their main active ingredient could be linked using their chemical profile by the similarity and correlation of two or more impurities found in the synthesized MDMA [212, 273].

In the present study the detected impurities were identified, using the EI mass spectra published in the literature was not high. The number of detected impurities depended on such factors as the purification of MDMA prior to tabletting of the tablets at the clandestine laboratories and the storage conditions of the tablets which might have altered some minor chemicals to new impurities [266] that were not reported in the literature. If MDMA is thoroughly purified after synthesis this decreases the number of available markers in the tablets making it sometimes difficult to determine the synthetic route for MDMA production [272].

There are four main chemical precursors that are normally used to synthesise MDMA. These are: safrole, isosafrole piperonal and 3,4-MDP2P [121, 273]. The precursors, especially isosafrole, piperonal and 3,4-MDP2P, were detected in all 22 tablets that were chemically profiled. All the four precursors, which are available commercially, are controlled by the UN (the 1988 UN Convention Against Illicit Traffic in Narcotic Drugs and Psychotropic Substances), the European Union [121] including the UK and Malta as described in Chapter 1, Section 1.5.1.

The precursors chemicals that were most often found together in the same chemical profile from the 22 MDMA tablets were: the two precursors chemicals piperonal and 3,4-MDP2P (31.8%, n = 7), and the three precursor chemicals isosafrole, piperonal and 3,4-MDP2P (27.3%, n = 6, possibly because both piperonal and 3,4-MDP2P can both be synthesised from isosafrole [128, 293]. However, it seems that 3,4-MDP2P was the precursor that was mostly used to synthesise the MDMA because it was found in 19 tablets (86.43%). Two other impurities, 3,4-(methylenedioxy)-phenylpropane and piperonyl alcohol, were detected in 5 tablets (22.7%). The former is formed by the chemical reduction of safrole or isosafrole [121], and the latter by the chemical reduction of piperonal [121]. Other impurities indicated the possibility that MDMA in the 22 tablets was synthesised by either the Leuckart or the reductive amination reactions.

The Leuckart reaction, which is the most usual route for the production of amphetamine type drugs, can also be used for the production of the methylenedioxy-substituted analogues [200]. Two impurities DMMDA and N-formyl which could be formed during the synthesis of MDMA by the Leuckart reaction were detected in 50% (n = 11) of the profiled tablets. Renton et al. [200] claimed that the presence of the impurity DMMDA in MDMA prepared by N-methylformamide / formic acid reaction was possibly caused as a result of N,N-dimethylformamide impurity in the N-methylformamide [200]. The other impurity, N-formyl-MDMA, which is considered as a Leuckart reaction marker, is formed during the reaction between 3,4-MDP2P, N-methylformamide and formic acid [123, 212]. The resulting N-formyl-MDMA is then converted to MDMA by refluxing using either a strong acid or base [294]. Thus the presence of DMMDA and N-formyl-MDMA in six batches indicates that the MDMA in these tablets was possibly synthesised by the Leuckart reaction. [200, 240, 273].

Another impurity N-acetyl MDMA was found in two of the analysed tablets (batches 18 and 28, white tablets with D&G and heart logo respectively), together with N-formyl-MDMA. The presence of the N-acetyl MDMA impurity was reported in two studies that were conducted in France between the years 1999 - 2000 [273] and in Australia on tablets seized in Macedonia between the years 2006 - 2007 [295]. The tablet samples from

Macedonia contained both N-acetyl-MDMA and N-formyl-MDMA [295]. It could thus be speculated that N-acetyl MDMA, which was found together with N-formyl-MDMA, is another impurity formed during the Leuckart synthesis of MDMA.

The synthesis of MDMA by reductive amination involves the use of 3,4-MDP2P and an amine under reducing conditions as described in Chapter 1, Section 1.5..2. The presence of the alcohol 3,4-methylenedioxyphenyl-2-propanol detected in half of the profiled MDMA tablets (n = 11, batches 17-19, 22-24, 27, 33, 40, 44-45) together with 3,4-MDP2P could be an indication that the reductive amination route was used [212, 273]. The alcohol could have been formed by the reduction of 3,4-MDP2P with excess reductant during the synthesis of MDMA as reported by Cheng et al. [212]. However, 3,4-methylenedioxyphenyl-2-propanol could have also be formed during the synthesis of MDMA by the bromopropane route [273]. Since no brominated impurity compounds were detected in the profiles where the compound 3,4-methylenedioxyphenyl-2-propanol was present, the probability was that the alcohol was produced during the synthesis of MDMA by reductive amination. Both N-formyl-MDMA, which is normally considered as Leuckart route marker for MDMA [200, 296] synthesis and DMMDA were also found as impurities in MDMA synthesised by reductive amination [272, 273]. The presence of DMMDA in MDMA impurity profile synthesised by reductive amination was detected in 5 chemically profiled tablets (22.7%). The presence of MDA as an impurity in the chemical profile of some (n = 5, 22.7%) of the tablets further confirmed the possibility that the MDMA in these tablets was synthesized by reductive amination. Both MDA and DMMDA impurities detected in some of the profiles of the tablets are normally produced by the ammonia impurity present in the methylamine during the reductive amination of 3,4-MDP2P [296]. Another impurity which was detected in the tablets (n = 5, 22.7%), which is normally also attributed to reductive amination, was N--methyl-(1,2-(methylenedioxy)-4-(1-ethyl-2-aminopropyl)benzene [297]. Thus from the chemical impurity profiling of the MDMA-only tablets from the 22 batches it was concluded that 13 tablets (59.1%) were probably synthesized using the reductive amination reaction, while 9 tablets were probably synthesized using the Leuckart reaction.

Two other compounds which were also detected in some of the tablets (n = 7, 31.8%) included palmitic and stearic acid, compounds which are normally used as lubricants [273] during tablet manufacture. The presence of cocaine in the chemical profile of two tablets (blue coloured with omega logo, batch number 6 and white coloured with heart logo, batch number 28 respectively) was found to be contact contamination. This was confirmed by analyzing two other tablets from the same batches where no traces of cocaine were detected.

In this study (Section 5.3.3, Experiment C) the comparison of the TIC profiles of the of the blue and green MDMA-only tablets with omega logo from batches 5 and 8 and the comparison of the TIC profiles of the two pink MDMA-only tablets with lacoste logo from batches 44 and 45 showed similarity between the impurities. The correlation between the impurities of the two pink tablets, shown in Figure 5.10 above, such as piperonal, 3,4-MDP2P, MDA, 3,4-methylenedioxy-phenyl-2-propanol and 3-methyl-6,7-methylenedioxy-3,4-dihydroisoquinolin-1(2H)-one, indicated that the method used to synthesise MDMA was the same, possibly reductive amination. The presence of the impurity 3-methyl-6,7-methylenedioxy-3,4-dihydroisoquinolin-1(2H)-one, detected in the same TIC profile (Figure 5.10) could be indicative that the used precursor 3,4-MDP2P was synthesised from piperonal via β-nitroisosafrole [272] (MDP-2-nitropropene). Other two detected peaks on the TIC profiles of the two tablets from batches 44 and 45, which were not synthetic impurities, were palmitic and stearic acids used as lubricants during tablets manufacture. Although there was some variability between the TIC profiles of the tablets from batches 44 and 45 respectively, this could have been caused by intra and inter-batches variability, of tablets. The similarity between the chemical profiles of these four tablets (blue and green tablets with omega logo and 2 pink tablets with lacoste logo) was indicative that the batches were possibly linked.

The overall results indicated that the difference in the precursor chemicals and the synthesis methods used to produce MDMA found in the analysed tablets provided different chromatographic profiles. It was also concluded that the MDMA in the tablets seized in Malta during the period of study (2006 – 2011) was predominately synthesised

by one of the different reductive amination reactions using the intermediate 3,4-MDP2P which was prepared from piperonal. This finding was consistent with the literature [209, 273].

In ***experiment (D)*** the use of FTIR transmittance spectroscopy was applied to determine the major excipients in the seized 'ecstasy' tablets. This approach which was novel, as far as it is known, have never been used to identify major excipients in 'ecstasy' tablets.

By FTIR transmittance analyses of 'ecstasy' tablets from 35 batches it was possible to identify four major excipients, which included the excipients lactose, sorbitol, DCP and starch and one minor excipient the lubricant magnesium stearate, which was present in most tablets (97.1%, n = 33). The presence of DCP and / or magnesium stearate in some of the tablets was confirmed by SEM/EDX analyses. The most commonly encountered excipient in the analysed 'ecstasy' tablets was lactose (34.3%, n =12), followed by sorbitol (25.7%, n = 9), DCP was found in 8 tablets and starch in 6 tablets. The finding of lactose and sorbitol as the most common excipients in the anlysed 'ecstasy' tablets was concurrent with results of another study [126]. It was noted that the excipient DCP was present in most of the tablets (7 out of 9 tablets containing other active ingredient instead of MDMA, batches 31, 32, 34, 35, 37-39) containing mCPP or caffeine as their main and only psychoactive substance.

The possibility of using FTIR transmittance spectroscopy method to distinguish between tablets from different batches was also investigated. The FTIR transmittance analyses of 'ecstasy' tablets produces spectra that represent the particular tablet composition as a whole. Therfore, it seemed possible that FTIR transmittance spectroscopy could be used to link or differentiate 'ecstasy' tablet from different batches by comparing FTIR peaks characteristics. This was partially confirmed from the comparison of the FTIR spectra of 'ecstasy' tablets containing MDMA. The FTIR spectra of MDMA-only tablets was fairly similar, the only noted difference was in the FTIR 'fingerprint' region when the composition of the tablets was different. It was also noted that when MDMA-only tablets

had similar physical features, such as colour, shape and logo, such as batches 5-7, 8-10, 11-13, 14-16, and 44-45, the FTIR 'fingerprint' region was similar. The similarity in the spectra of tablets with similar physical features could be attributed to the powdering of the tablets, thus overcoming any difference that might have been in the homogenity, prior to the FTIR transmittance analyses.

The FTIR transmittance spectra of the blue and green tablets with the omega logo, batches 5-10, and the pink tablets with the lacoste logo, batches 44-45, taken from different batches and seized on different occassions, were found to be very similar. Hence it was speculated that the blue and green tablets with the omega logo like the pink tablets with lacoste logo had a common origin. It was also speculated that the two batches of pink tablets with the lacoste logo might have been a single batch which was divided for distribution. This was concluded from FTIR spectra and the results from the physical and chemical characterisation.

Overall it was found that the FTIR transmittance spectroscopy method could be used to determine major illicit tablet excipients. Moreover, the results obtained from comparing the FTIR peaks characteristics indicated that this method could be used with other physical and characterisation methods, to link or differentiate between 'ecstasy' tablets from different batches.

In ***experiment (E)*** the use SEM/EDX analyses of 'ecstasy' tablets was to determine the elements present in the tablets and to use the detected elements to inorganically profile the tablets. This was a new approach of elemental profiling of 'ecstasy' tablets. Ten ions of elements (which were Na, Mg, Al, Si, P, S, Cl, K, Ca and Ti) were detected. The most common elements that were detected on the 37 'ecstasy' tablets from the batches were the Cl (97.3%, n = 36), followed by Mg (91.9% n = 34) and Si (83.8%, n = 31). However, there was variation in the presence of excipients in different 'ecstasy' tablets, which together with the elements present and their concentration provided the possibility of linking or discriminating between tablets. When a sufficient number of elements were

detected, as was the case for the tablets from batches 34-39, where 10 elements where detected (Na, Mg, Al, Si, P, S, Cl K, Ca, and Ti) or the percentage weight of the respective elements was different it was possible to establish an elemental profile and differentiate between samples. It has also been shown that the inorganic profile and the percentage weight alone in some instances was not enough to discriminate between samples. This has been shown above were tablets from batches 11, 17, 26-28 and 33 had similar elemental profile (Mg, Si and Cl) and percentage weight. This could be an indication that if inorganic profiling, using SEM/EDX analyses, is taken alone a false positive link could occur between two samples. Thus it could be that elemental analysis would not be enough to discriminate or link 'ecstasy' tablets from different seizures and that additional information, such as physical and chemical profiling, would be necessary to interpret the results.

The elemental ions detected by SEM/EDX analyses on the surface of the tablets could most be explained by the fact that these elements could be found in excipients used in the manufacture of 'ecstasy' tablets [144]. However, no elemental ions were detected on one of the tablets, blue coloured with a heart shape containing an anabolic steroid (batch 30), indicating that the excipients used in this tablet were all organic. Although excipients make a big part of the 'ecstasy' tablets there is very little information about these in the literature. This was the first study that has investigated the use of elemental ions from excipients for 'ecstasy' tablet profiling.

The presence of the elements Mg, Ca, Si, which were found in most of the tablets (83.8%, n = 31) was probably from glidants used in the tablets such as magnesium carbonate [298], talc (hydrated magnesium silicate) and silicone dioxide (fumed silica) [299, 300]. Glidants are used in tablets to improve the flow property of the formulation by reducing friction and cohesion [298, 299]. Some of the detected elemental ions included the elements Na, Ca, the presence of S for the sulphate and the presence of P for the phosphates [144] which have been due to the presence of excipients, used to increase the bulk content of the tablet (e.g. both magnesium and calcium carbonate [301], calcium

sulphate and dibasic calcium phosphate [302], (dibasic calcium phosphate was detected by FTIR was confirmed by SEM/EDX). Other detected elements included the Na, Mg, Al, Ca which have originated from the lubricants Mg, Al and Ca stearates (Mg stearate confirmed by FTIR) and Na benzoate [144, 299, 300]. The lubricants are used in tablets to reduce the friction between the tablet and the die during the tablet ejection from the die [299]. The elemental ions detected on the surface of most (94.6%, n =35) of the 'ecstasy' tablets were from the excipients (glidants, diluents and lubricants) which are concurrently used during tablet manufacture. Organic excipients (i.e. glidants, diluents and lubricants) were used in the tablets where no elemental ions were detected (e.g. batch 30 and batch 40).

The detection of the element Ti (batches 34, 35, 37 and 38) was probably due to the use of a 'whitener' in the tablets [300]. The detected elemental ion Cl was mainly due to hydrochloride salt of the active ingredients present. It is claimed that in large scale production of MDMA-HCl high yields are obtained by the use of gaseous HCl instead of the acid and that crytallisation is performed in closed vessels at low temperatures [179].

Overall the number of detected elemental ions, a total of ten ions, mainly from the excipients of the analysed 'ecstasy' tablets was rather low when the SEM/EDX profiling method was used. This method, which is semi-quantitative, could sometimes not be enough to link or discriminate between samples of 'ecstasy' tablets; however, some batches of tablets could still be differentiated by SEM/EDX profiling.

Finally, this study has demonstrated that by using chemical characterisation it was possible to determine if 'ecstasy' tablets from different police seizures could be linked or differentiated. This was achieved by five experiments and examining the results for similarities or differences in the psychoactive substances and their amounts in the tablets, the synthetic impurities and the enantiomer ratio for MDMA-only tablets, the major excipients and the elemental profile of the tablets, irrespective of their physical features. While most (80.0%, n = 36) of tablets could be differentiated by chemical characterisation, 14 batches were found to be linked (batches 5-7, 8-10, 11-13, 14-16, 44-

45). This study also evaluated ***hypothesis (4)*** that:

> ***The chemical composition of different batches of 'ecstasy' tablets seized on different occasions in Malta during 2006 – 2011 will be significantly different from each other.***

which was not verified

Chapter 6

ELECTRONIC DANCE MUSIC EVENTS: ILLICIT SUBSTANCES AND BEHAVIOUR OF PARTICIPANTS

This chapter consists of two studies which deal with: (a) seizures of illicit substances from electronic dance music (EDM) events in Malta over a six year (2006 - 2011) period by Malta Police Drug Squad and (b) a study which investigated the behaviour of participants at an EDM event in Malta during the summer of 2010.

6a Seizures of illicit tablets and substances at major electronic dance music (EDM) events in Malta.

The extent of drug use at electronic dance music (EDM) events in Malta tends to be anecdotal accounts of use and little formal investigation has been made. This research was to investigate substances being consumed at a party. Young people attend these dance venues to socialise and dance, and the use of both legal and illicit substances during this time become necessary [41]. In Malta plain clothes policemen are normally present during EDM events. They aim to stop and search young people, while entering and during the party, for illicit substances. This section will describe the results of analyses of substances seized over a six years period (2006 - 2011) from eighteen parties.

6a.1 Introduction

Modern late-night EDM parties, together with drug use, were imported to major European cities mainly from Ibiza [303]. As already discussed in Chapter 1, Section 1.1.2, these parties were originally rave parties and where held in secret warehouses, and then began to be highly promoted and organized in popular venues such as bars and dance clubs [304]. One of the factors that caused this shift was legislative control, which

mainly forbade raves from being organised in clandestine spaces. This pushed raves towards partnership with legal clubs and consequently raves became commercialised EDM parties and part of the leisure industry. The change in raves to commercialised EDM parties brought with it a change in substance use [305], where alcohol became part of the drug mix [304]. The same happened in Malta where illegal rave parties were pushed to legal main stream night club and dance setting, where alcohol, 'ecstasy' and cocaine were used [48].

A study was conducted to investigate the illicit substances confiscated by the police, from partygoers at EDM events. The information gathered from the analyses of the illicit tablets and substances would provide more accurate information on the exact contents of these substances to healthcare professionals, since this information would not depend on what users thought these substances contain. The study also examined the visual and measurable features together with the dose of the seized 'ecstasy' tablets from EDM events and compared the data with the data from the 45 batches and also attempted to test the ***hypothesis (5)*** that:

> ***Those 'ecstasy' tablets seized by the police at EDM events in Malta will match batches seized on different occasions in Malta.***

6a.2 Methodology

6a.2.1 Drugs seized at EDM parties

Ramsey et al**.** [306] developed a method to monitor illicit substance use at EDM parties in the UK, which involved the analyses of substances discarded in amnesty bins by clubbers before entering the party [306]. In this study substances were confiscated by the police from partygoers during major EDM parties and not freely volunteered by the clubbers themselves. These were seizures confiscated by the police during eighteen large open air EDM parties over a six year period (2006 - 2011), that were examined and analysed.

In Malta, during major EDM parties, the police stop and search approximately sixty people (range from 50 to 80 persons) and always detain a number (average 10 persons, range from 5 to 13) of partygoers (N. Harrison, 2006, personal communication, Assistant Commissioner, Malta Police, telephone conversation, 10 January) who are found in possession of substance/s suspected to be illegal drugs. Seized drugs found in possession of each party attendee would be individually packed (considered as one seizure) by the Drug Squad Police at the party, and sent for examination and analysis at the Forensic Laboratory by order of the Inquiring Magistrate. The contents of each seizure, contained in separate plastic bags, were individually examined and analysed by the researcher.

6a.2.3.1 Tablet and substance examinations

The visual characteristics of the 'ecstasy' tablets were noted and the physical characteristics, mass, diameter and thickness, were measured as described in Sections 2.2.1.1 and 2.2.1.3 and analysed using colour tests, TLC and GC-MS analyses as described in Sections 2.2.3.1 and 2.2.4.1. The identification of MDMA tablets were specified by TFAA derivatisation and quantified as described in Sections 2.2.3.1. For the powders and plants and cannabis-like material, a methanoic extract was prepared with a final concentration of approximately 1mg mL^{-1}. The powders were assumed to be 50% pure, while the plants and cannabis-like material was assumed to be 10% pure. For liquid suspected to be gamma-butyrolactone (GBL), a 0.1% solution in chloroform was prepared.

6a.3 Results

6a.3.1 Monitoring of seized tablets and substances

A total of 172 seizures were investigated, which contained 238 separate items: 75 tablets (31.5%), 83 powders (34.9%) (white and brown), 79 plants and cannabis-like material (33.2%) and 1 (0.4%) small amount of transparent liquid. The drug seizures over the 6 year period from 18 large open air "commercial" EDM parties are shown in Table 6a.1.

Most of the seizures (80.2%, n = 138) contained one substance, while the rest contained two or more drugs.

Table 6a.1 Seizures from EDM parties in Malta during a 6 year period (2006 - 2011).

Year	EDM Party	Seizures	Year	EDM Party	Seizures	Year	EDM Party	Seizures
2006	1	12	2008	7	10	2010	13	10
	2	9		8	6		14	9
	3	11		9	9		15	11
	Total	**32**		10	6		**Total**	**30**
2007	4	10		**Total**	**31**	2011	16	13
	5	8	2009	11	10		17	11
	6	7		12	11		18	9
	Total	**25**		**Total**	**21**		**Total**	**33**

A total of 75 tablets were found in nearly one third of the seizures (29.7%, n = 51). Most, (94.7%, n = 71) of the tablets had logos and white (74.7%, n = 56), a much smaller number were blue (10.7%, n = 8) or pink (9.3%, n = 7) and 2 tablets were coloured green and yellow respectively. Table 6a.2 shows the substances detected in the 172 seizures.

From the GC-MS analyses it was found that nearly a quarter, (23.8%, n = 41), of the seizures (n = 172) contained MDMA tablets and that 61 (85.9%) out of the 71 'ecstasy' tablets contained MDMA (Table 6a.3 below). In most (95.1%, n = 39) of the seizures where MDMA was the only psychoactive substance found in the tablets, one to two tablets were involved.

The MDMA tablets (n = 61) were all round with most, (77.1%, n = 47), having a flat side, more than half of the tablets had a breakline, (54.1%, n = 33), and were coloured

Table 6a.2 A total of 238 substance items were detected in the 172 seizures, which were confiscated during 18 major EDM parties (% represents the percentage of the specific item with respect to the total number of items found, 238 items, and N is number of specific items found).

Compound	%	(N)
Tablets		
MDMA	**25.6**	**(61)**
BZP	02.5	(06)
Caffeine	00.8	(02)
mCPP	00.8	(02)
Dihydrocodeine	00.8	(02)
Promethazine	00.4	(01)
Sildenafil citrate (Viagra)	00.4	(01)
Powders		
Cocaine	**18.9**	**(45)**
Unidentified	05.5	(13)
Mephedrone	03.4	(08)
Heroin	02.1	(05)
Caffeine (range 0.2-0.4 g)	01.7	(04)
Paracetamol (range 0.3–0.5 g)	01.7	(04)
Lignocaine (mean 0.2 g)	00.8	(02)
Amphetamine (0.4 g)	00.4	(01)
Ketamine (0.2 g)	00.4	(01)
Plants and cannabis-like material		
Cannabis items	**31.9**	**(76)**
1-pentyl-3-(1-naphthoyl)indole (JWH-018) (range 0.98-1.2 g)	01.3	(03)
Liquids		
Gamma-butyrolactone (GBL) (3 mL)	00.4	(01)

white (68%, n = 42, the 14 other tablets had other active ingredients). The MDMA tablets had logos, the most common logo on the tablets was the euro symbol with (9.8%, n = 6), followed by smiley, (8.2%, n = 5), heart, D&G and rolex, with (6.6%, n = 4 each) tablets (Tables from 6a.3 to 6a.8).

The means of the measurable features including the dose of the seized 61 MDMA tablets were: mean mass 228 mg (SD 25.56, range 184 – 276 mg), mean diameter was 8.00 mm (SD 0.77, range 7.01 – 9.67 mm), mean thickness was 3.89 mm (SD 0.72, range 2.75 – 5.39 mm) and mean dose was 54.4 mg (SD 14.10, range 12.9 – 75.2 mg). Nearly

Table 6a.3 'Ecstasy' tablets containing MDMA found in 13 seizures in 2006.

Tablet	Breakline	Logo	Mass (mg)	Diameter (mm)	Thickness (mm)	Dose (mg)
	yes	heart	266	8.71	4.62	75.2
	yes	tulip	248 252	7.13 7.18	4.92 5.20	70.3 74.6
	yes	euro	249	7.13	4.74	73.7
	yes	diamond	234	9.44	3.31	42.2
	no	smiley	211 213 218	7.89 7.92 8.21	3.31 3.31 3.32	61.6 62.7 65.1
	no	shell	217	7.06	4.82	53.4
	yes	mitsubischi	249	7.06	4.45	68.1
	yes	euro	230	7.11	4.69	50.4
	yes	star	264 266	7.32 7.46	5.02 5.03	65.3 66.7
	yes	yin-yang	184	8.02	3.34	59.8
	no	cherries	247	7.21	5.22	68.6
	yes	mitsubischi	256 258	7.86 8.32	4.72 4.30	71.4 73.1
	no	armani	187	7.12	3.51	40.3
		mean values and SD	**236.1 (25.8)**	**7.68 (0.68)**	**4.32 (0.75)**	**63.5 (10.60)**

three quarters of the tablets, (72.1%, n = 44), had a mass which ranged between 200 – 250 mg (typically ≈ 240 mg, 16.4%, n = 10). The majority (95.1%, n = 58) of MDMA

tablets had diameter which could be grouped in three classes, 7, 8, and 9 mm (typically 8 mm, 39.3%, n = 24), thickness which could be grouped in three classes of 3, 4, and 5 mm, (98.4%, n = 60, typically 4 mm, 39.3%, n = 24).

The mean MDMA content in the ecstasy tablets over the 6 year period ranged between a maximum of 63.5 mg in 2006 and a minimum of 38.8 mg in 2008. There was a significant decrease ($p < 0.05$, one-way ANOVA) in the mean MDMA content when the year 2008 (mean 38.8 mg) was compared with years 2006 (mean 63.5 mg), 2007 (mean 56.6 mg), 2010 (mean 57.9 mg) and 2011 (mean 64.2 mg) and when the year 2009 (mean 46.9 mg) was compared with years 2006 and 2011 respectively (Tables from 6a.3 to 6a.8).

In 2006 there were thirteen seizures which contained 18 MDMA tablets (Table 6a.3). The tablets in four of the seizures, which contained more than one tablet in each seizure,

Table 6a.4 'Ecstasy' tablets containing MDMA found in 7 seizures in 2007.

Tablet	Breakline	Logo	Mass (mg)	Diameter (mm)	Thickness (mm)	Dose (mg)
	**no**	**D&G**	**194** **201**	**7.01** **7.02**	**4.08** **4.13**	**53.9** **57.1**
	yes	shark	283	9.04	3.75	68.1
	yes	diamond	265	8.54	4.03	63.7
	**no**	**heart**	**196** **202**	**7.03** **7.09**	**4.38** **4.38**	**57.2** **60.1**
	**no**	**euro**	**210** **214**	**8.08** **8.11**	**4.30** **4.39**	**56.4** **57.7**
	**no**	**kangaroo**	**240**	**8.09**	**3.57**	**47.9**
	yes	twins	193	7.11	3.62	44.2
Tablets		**mean values and SD**	**219.8 (31.97)**	**7.71 (0.75)**	**4.06 (0.32)**	**56.6 (6.94)**

had tablets which were very similar in both their physical measurable feature and the dose (white with tulip logo, 2 tablets, white with smiley logo, 3 tablets, white with star logo, 2 tablets, and white with mitsubishi logo, 2 tablets, Table 6a.3).

In 2007 there were seven seizures which contained ten MDMA tablets. The tablets in three of the seizures, which contained more than one tablet in the seizure, had tablets which were very similar in both the physical measurable features and the dose (white with D&G logo, 2 tablets, white with heart logo, 2 tablets and white with euro logo, 2 tablets, Table 6a.4). The two tablets with euro logo seized in 2007 were not found to be related with the euro tablet seized in 2006 because both the measurable features and the dose of MDMA were different.

In 2008 there were eight seizures which contained twelve MDMA tablets (Table 6a.5). The tablets in four of the seizures, which contained more than one tablet, had tablets

Table 6a.5 'Ecstasy' tablets containing MDMA found in 8 seizures in 2008.

Tablet	Breakline	Logo	Mass (mg)	Diameter (mm)	Thickness (mm)	Dose (mg)
	**no**	**D&G**	**194** **197**	**7.03** **7.04**	**3.91** **3.94**	**49.7** **51.2**
	yes	fish	221	7.86	4.34	54.7
	**yes**	**heart**	**236**	**8.39**	**4.94**	**61.6**
	**no**	**euro**	**202** **203**	**7.13** **7.13**	**3.59** **3.59**	**13.4** **12.9**
	**yes**	**question mark**	**242**	**7.12**	**5.39**	**40.3**
	no	butterfly	199 201	8.04 8.12	3.41 3.67	48.5 50.3
	**no**	**smiley**	**211** **213**	**8.21** **8.22**	**3.29** **3.30**	**19.9** **20.5**
	**yes**	**star**	**193**	**8.10**	**3.44**	**42.6**
Tablets		**mean values and SD**	**209.3 (16.1)**	**7.70 (0.55)**	**3.90 (0.67)**	**38.8 (17.31)**

which were very similar in both the physical measurable features and the dose (white with D&G logo, 2 tablets, white with heart logo, 2 tablets, violet with butterfly logo, 2 tablets and pink with lacoste logo, 2 tablets, Table 6a.5). The two tablets with the D&G logo seized in 2008 were found to have similar measurable features and dose with two other D&G tablets that were seized in 2007 (Tables 6a.4 and 6a.5). However, the measurable features and the dose of the two tablets with the euro logo seized in 2008 (Table 6a.5) were not found to be related with the euro tablets seized in 2006 (Table 6a.3) and 2007 (Table 6a.4).

In 2009 there were six seizures which contained ten MDMA tablets (Table 6a.6). Two of the seizures, which contained more than one tablet in each seizures, had tablets which were very similar in both the physical measurable features and the dose (white with rolex crown logo, 4 tablets, and white with versace logo, 2 tablets, Table 6a.6). The other tablets had different physical features and dose.

Table 6a.6 'Ecstasy' tablets containing MDMA found in 6 seizures in 2009.

Tablet	Breakline	Logo	Mass (mg)	Diameter (mm)	Thickness (mm)	Dose (mg)
	yes	rolex	238 239 243 248	8.51 8.73 9.45 9.67	2.76 2.87 3.09 3.16	40.4 42.6 47.1 48.3
	yes	volkswagen - VM	234	8.51	3.34	38.9
	yes	smurf	224	7.93	4.47	60.3
	yes	crown	237	8.69	3.01	45.7
	**yes**	**versace**	**268** **276**	**8.20** **8.21**	**4.34** **4.38**	**50.3** **51.8**
	no	cross	191	7.89	3.43	43.4
Tablets		**mean values and SD**	**239.8 (16.60)**	**8.58 (0.59)**	**3.49 (0.66)**	**46.9 (6.29)**

In 2010 there were three seizures which contained five MDMA tablets (Table 6a.7). The tablets in two of the seizures, which contained more than one tablet in the seizure, had tablets which were very similar in both the physical measurable features and the dose (white with dollar logo and white with $E=mc^2$ logo, 2 tablets, Table 6a.7). The other tablet (logo motorala) had different physical features and dose.

Table 6a.7 'Ecstasy' tablets containing MDMA found in 3 seizures in 2010.

Tablet	Breakline	Logo	Mass (mg)	Diameter (mm)	Thickness (mm)	Dose (mg)
	yes	Dollar	216 220	7.58 7.83	4.21 4.45	57.6 59.8
	no	motorola	226	7.81	4.87	67.8
	**no**	**$E=mc^2$**	**218** **209**	**9.06** **9.04**	**3.01** **2.75**	**53.7** **50.6**
Tablets		**mean values and SD**	**217.8 (6.18)**	**8.26 (0.72)**	**3.86 (0.93)**	**57.9 (6.57)**

In 2011 there were four seizures which contained six MDMA tablets (Table 6a.8). The tablets in two of the seizures, which contained more than one tablet in each seizure, had tablets which were very similar in both the physical measurable features and the dose

Table 6a.8 'Ecstasy' tablets containing MDMA found in 4 seizures in 2011.

Tablet	Breakline	Logo	Mass (mg)	Diameter (mm)	Thickness (mm)	Dose (mg)
	**no**	**$E=mc^2$**	**237**	**9.09**	**3.06**	**58.1**
	**cross**	**route 66**	**238** **224**	**9.08** **9.10**	**3.27** **3.18**	**66.2** **62.4**
	yes	superman	272	7.64	4.89	71.2
	**cross**	**lacoste**	**252** **241**	**9.20** **9.21**	**3.30** **3.51**	**65.3** **61.9**
Tablets		**mean values and SD**	**244 (16.38)**	**8.89 (0.61)**	**3.54 (0.68)**	**64.2 (4.47)**

(white with route 66 logo and pink with lacoste logo, 2 tablets, Table 6a.8). It was noted that the tablet with the $E=mc^2$ logo seized in 2011 had fairly similar measurable features and dosage to two tablets with the same logo seized in 2010 (Table 6a.7).

'Ecstasy' containing other stimulants and other tablets

The GC-MS analyses of 10 'ecstasy' tablets from 8 seizures (4.7% of the total seizures, n = 172) were found to contain a psychoactive substances other than MDMA (Table 6a.9 below). In six of the tablets, having a diamond shape, BZP was detected. The means of the measurable features and dose of the BZP tablets were: mean mass was 438 mg (SD 14.19, range 417 – 450 mg), mean width was 8.18 mm (SD 0.13, range 8.00 – 8.40 mm),

Table 6a.9 'Ecstasy' tablets containing other psychoactive substances.

Tablet	Year	Breakline	logo	Mass (mg)	Diameter (mm)	Thickness (mm)	Active Ingredient	Dose (mg)
	2006	**no**	**no**	**450**	**l – 11.2 w – 8.0**	**6.22**	**BZP**	**214**
				447	**l – 11.3 w – 8.1**	**6.14**		**211**
				448	**l – 11.3 w – 8.2**	**6.25**		**212**
				423	**l – 11.1 w – 8.2**	**5.98**		**201**
				417	**l – 11.3 w – 8.2**	**6.08**		**199**
	2007			**442**	**l – 11.4 w – 8.4**	**5.93**		**207**
	2008	**no**	**tulip**	**209**	**7.12**	**3.23**	**mCPP**	**60.3**
	2010	yes	apple	228 224	7.89 7.80	3.91 3.78	Caffeine	19.9 18.3
	2011	no	bird	221	6.89	4.04	mCPP	63.6

mean length was 11.27 mm (SD 0.10, range 11.1 – 11.40 mm), the mean thickness was 6.10 mm (SD 0.013, range 5.93 – 6.25 mm) and the mean dose was 207.3 mg (SD 6.16,

range 199 – 214 mg). Two other tablets, one with the tulip and another with bird logos, contained mCPP and another two tablets, with the apple logo, contained caffeine. Three of the four types of 'ecstasy' tablets found had logos and only one type, tablet with apple logo, had a breakline. The tablets were all white coloured and had a round shape with the exception of six tablets which had a diamond shape. The means of the measurable features of the 4 round tablets were: mean mass was 220.5 mg (SD 8.19, range 209 – 228 mg), mean diameter was 7.43 mm (SD 0.50, range 6.89 – 7.89 mm) and the mean thickness was 3.74 mm (SD 0.36, range 3.23 – 4.04 mm). Moreover, the mean dosage of the mCPP tablets was 62.0 mg and the mean dosage for the caffeine tablets was 19.1 mg.

Some of the 'ecstasy' tablets that were seized at the EDM events during 2006 – 2011, shown in bold in Tables 6a.4 to 6a.9 were found to have very similar physical measurable features and dosage with seizures containing ≥ 100 MDMA tablets which were examined, analysed and described in Chapters 3 and 5 respectively (Table 6a.10). Moreover, the tablet seizures also included three tablets usually obtained by prescription (promethazine, dihydrocodeine and a counterfeit sildenafil citrate tablet).

6a.3.1.2 Powders

Of the 83 doses of powder, found in 77 seizures, more than one third (37.3%, n = 31) were individually wrapped in paper sachets while the rest, 53 doses, were individually sealed in small plastic bags. Cocaine was the substance most commonly found in powders, (54.2%, n = 45, typical mass – 0.5 g, range 0.4 - 1.2 g), followed by mephedrone (9.6%, n = 8, typical mass 0.2 g, range – 0.1 - 0.4 g) and heroin (6.0%, n = 5 and mass range – 0.06 - 0.1 g). Other substances found in the powders included amphetamine powder (n = 1, 0.1 g), and caffeine (n = 4), paracetamol (n = 4), lignocaine, n = 1) and ketamine (n = 1 scheduled under Maltese drug laws). Cocaine powder was also found to contain lignocaine (6.7%, n = 3) and phenacetin (68.9%, n = 31). In 13 powders (15.6%, typical mass – 0.5 g, range – 0.8 -2.1 g), which were white in colour (76.9%, n = 10), no illegal or pharmaceutical substances were detected.

Table 6a.10 MDMA tablets seized at EDM parties which had similar physical features (visual and measurable) and dose to the batches of tablets.

Tablet / Batch	Year	Breakline	Logo	Mass (mg)	Diameter (mm)	Thickness (mm)	A.I.	Dose (mg)
	2006 / 2007	no	no	438	/	6.10	BZP	207.3
Batch 2	2006	no	no	432	/	6.10	BZP	206.4
	2007	no	euro	210 214	8.08 8.11	4.30 4.39	MDMA	56.4 57.7
Batch 17	2007	no	euro	211	8.09	4.37	MDMA	57.1
	2007	no	D&G	194 201	7.01 7.02	4.08 4.13	MDMA	53.9 57.1
Batch 18	2007	no	D&G	200	7.02	4.13	MDMA	57.2
	2007	no	heart	196 202	7.03 7.09	4.38 4.38	MDMA	57.2 60.1
Batch 19	2007	no	heart	202	7.10	4.38	MDMA	60.6
	2007	no	kangaroo	240	8.09	3.57	MDMA	47.9
Batch 21	2007	no	kangaroo	241	8.09	3.58	MDMA	48.8
	2008	no	euro	202 203	7.13 7.13	3.59 3.59	MDMA	13.4 12.9
Batch 24	2008	no	euro	202	7.13	3.59	MDMA	12.9
	2008	yes	star	193	8.10	3.44	MDMA	42.6
Batch 25	2008	yes	star	194	8.10	3.45	MDMA	43.5
	2008	no	smiley	211 213	8.21 8.22	3.29 3.30	MDMA	19.9 20.5
Batch 26	2008	no	smiley	209	8.20	3.28	MDMA	19.6
	2008	yes	question mark	242	7.12	5.39	MDMA	40.3
Batch 27	2008	yes	question mark	258	7.11	5.34	MDMA	43.8
	2008	yes	heart	236	8.39	4.94	MDMA	61.6
Batch 28	2008	yes	heart	236	8.40	5.08	MDMA	62.8
	2008	no	tulip	209	7.12	3.23	mCPP	60.3
Batch 31	2008	no	tulip	206	7.10	3.20	mCPP	58.5
	2009	yes	versace	268 276	8.20 8.21	4.34 4.38	MDMA	50.3 51.8
Batch 33	2009	yes	versace	274	8.19	4.39	MDMA	57.5
	2010 / 2011	no	$E=mc^2$	218 209 237	9.06 9.04 9.09	3.01 2.75 3.06	MDMA	53.7 50.6 58.1
Batch 40	2010	no	$E=mc^2$	237	9.09	3.01	MDMA	60.2
	2011	cross	route 66	238 224	9.08 9.10	3.27 3.18	MDMA	66.2 62.4
Batch 41	2010	cross	route 66	246	9.08	3.36	MDMA	69.3
	2011	cross	lacoste	252 241	9.20 9.21	3.30 3.51	MDMA	65.3 61.9
Batches 44 and 45	2011	cross	lacoste	249 248	9.20 9.20	3.48 3.48	MDMA	64.3 65.3

Key: For the batches the numbers given are the means.

6a.3.1.3 Plants and cannabis-like material

From the 76 cannabis items 24 (31.6%) were cannabis joints, while 28 (36.8%) small packets of tobacco were found to be mixed with herbal cannabis (marijuana – 7.1%, n = 13) or cannabis resin (hashish – 19.7%, n = 15). Other cannabis material found was crushed marijuana, (14.5%, n = 11, typical mass of 1 g, range 0.89 – 2 g), which were normally found in small plastic bags, and small pieces of hashish, (17.1%, n =13, typical mass of 1 g, range 0.76 - 1.5 g), where some were wrapped in aluminum foil. Furthermore, three samples of plant-like material, similar to marijuana from three different seizures, contained the substance naphthalen-1-yl-(1-pentylindol-3-yl)methanone (JWH-018), a 'synthetic cannabinoid'.

6a.3.1.4 Liquids

One of the seizures contained one small plastic bottle, in the form of a fish, which contained 3 mL of transparent liquid. The liquid was found to contain the substance GBL.

6a.3.2 Illegal drug seizures and drug combinations

The three major illicit drugs found in the seizures were the cannabis items, found in 76 (44.2%) seizures, 'ecstasy' tablets, 48 (27.9%) and cocaine, 45 (26.2%). The graph below (Figure 6a.1) gives an overview of the trends in the seizures of the major illegal drugs during major EDM events in Malta over the 6 year period.

There was a significant decrease ($p < 0.05$, one-way ANOVA) in the seizures of 'ecstasy' tablets by the police from EDM parties during 2010 and 2011, as compared to 2006, from 48.5% to 16.7% in 2010 and 15.2% in 2011 respectively. Conversely, there was a significant increase ($p < 0.05$, one-way ANOVA) in the seizure of cocaine from 15.2% in 2006 to 36.4% in 2011.

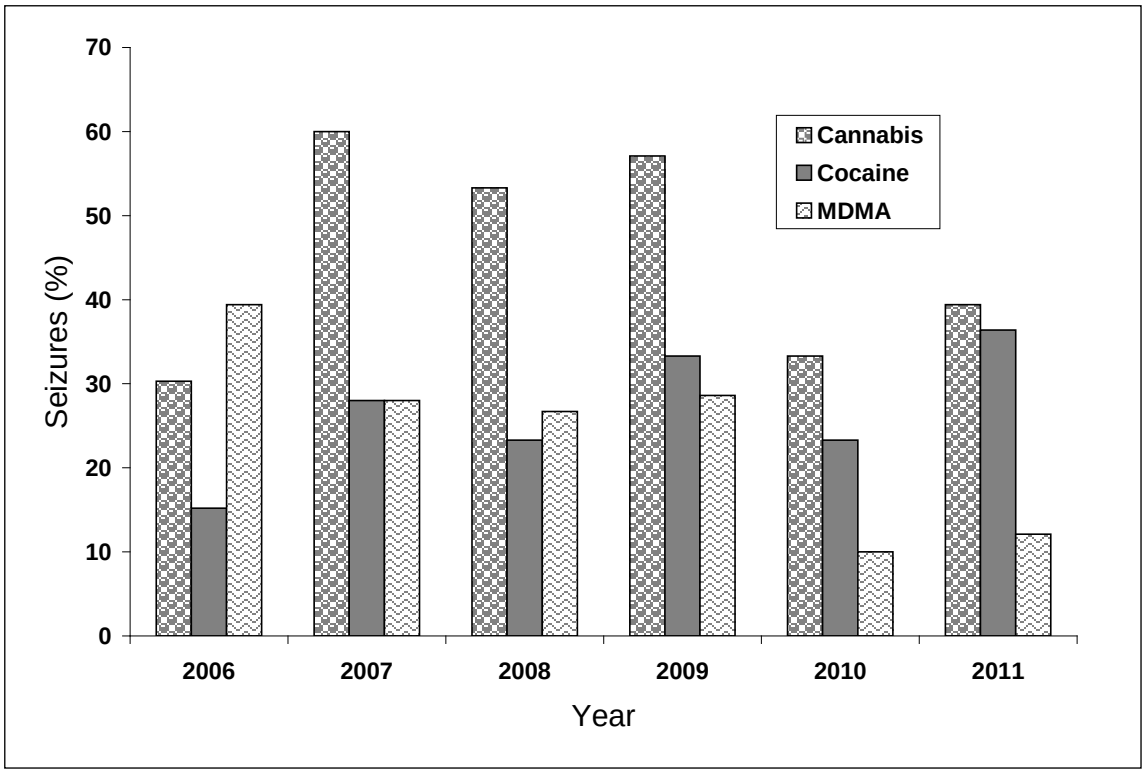


Figure 6a.1 Percentage seizures of cannabis items, cocaine and 'ecstasy' tablets containing MDMA over a 6 year period (2006 – 2011).

Confiscated 'ecstasy' tablets, containing MDMA and other psychoactive substances, were mostly found alone in the seizures (23.2%, n = 23). However, when 'ecstasy' tablets were found with other substances in the seizures, the most common were cannabis items (37.0%, n = 10) and to a lesser extent cocaine (11.1%, n = 3). When cocaine was found together with another substance in the seizure, the most common were the cannabis items (40.7%, n = 11). There was a significant difference ($p < 0.01$, one-way ANOVA) between the combinations of 'ecstasy' with cocaine or cannabis (54.2%, n = 13) and cannabis with cocaine or 'ecstasy' (87.5%, n = 21). Thus the combination of cannabis with either cocaine or 'ecstasy 'was significantly more likely than the use of 'ecstasy' with either cocaine or cannabis.

6a.4 Discussion

The aim of this study was to investigate illicit substance, with special attention to

'ecstasy' tablets confiscated by the police from major open air "commercial" EDM parties in Malta over a 6 year period from 2006 to 2011. The 'ecstasy' tablets found in the seizures were compared with the batches seized during 2006 – 2011. A total of 172 seizures containing 238 separate items, were examined. The items consisted of 75 (31.5%) tablets, 83 (34.9%) powders, 79 (33.2%) crushed plants and cannabis like material and 1 (0.4%) small amount of transparent liquid. The indications from these seizures were that the cannabis items were the most confiscated from major EDM events (44.2%, n = 76 seizures), followed by 'ecstasy' tablets (27.9%, n = 48 seizures) and cocaine (26.2%, n = 45 seizures).

Most (85.9%, 61 out of 71 tablets) of the 'ecstasy' tablets confiscated from the EDM parties contained MDMA. The MDMA tablets confiscated at EDM events had a mass which ranged between 200 - 250 mg (72.1%, n = 44). The majority, (95.1%, n = 58), of the tablets had diameters which could be grouped in three classes, 7, 8, and 9 mm, and thickness which could be grouped in three classes of 3, 4, and 5 mm, (98.4%, n = 60 tablets). The physical features, both visual and measurable, of the confiscated MDMA tablets were very similar to the physical features of 'ecstasy' tablets described in Chapter 3 and were similar to 'ecstasy' tablets seized in other countries, such as Israel [139], Finland, Netherlands, Czech Republic, France, Germany, Switzerland and USA [181] and Switzerland [214]. A total 30 tablets (42.3% of the total 'ecstasy' tablets) confiscated at EDM parties over the six years period had similar visual and measurable features (mass, diameter and thickness) and dosage to tablets from 16 out of the 45 batches (batches 2, 17-19, 21, 24-28, 31, 33, 40, 41, 44 and 45) described in Chapter 3. The 16 batches of tablets were seized during the same year when the 'ecstasy' tablets were confiscated from the EDM parties. Moreover, 41 (57.7% of the total tablets) 'ecstasy' tablets, with 29 different logos and colours, confiscated at the EDM parties during the six year period, did not match any of the tablets from the 45 batches. Thus ***hypothesis (5)*** that:

> ***Those 'ecstasy' tablets seized by the police at EDM events in Malta will match batches seized on different occasions in Malta.***

was not verified.

The mean MDMA content of the analysed 'ecstasy' tablets was found to have decreased from 63.5 mg in 2006 to 38.8 mg in 2008 and started to increase again in 2009 from 46.9 mg to 64.6 mg in 2011. The same happened in the UK where the mean MDMA base content of the 'ecstasy' tablets decreased from 64.5 mg in 2003 to 33.1 mg in 2008 and started to increase again in 2009 to 43.5 mg and continued to increase to 49.0 mg in 2010 [307, 308]. These results from Malta and UK are consistent with what was reported in the UN 2012 World Drug Report that "during the second half of the 2000s the decline in the availability of the main precursor of "ecstasy" 3,4-MDP2P led to a shortage of MDMA" [309].

According to the same report, producers had to reduce the MDMA content in "ecstasy" tablets and "use various other substances to compensate" [309] for the decrease in MDMA. These substances included the use of piperazines and caffeine and in fact these were also found in 'ecstasy' tablets seized in Malta from partygoers during the period 2006-2011. However, as the piperazines, BZP, mCPP and its isomers, were scheduled in Maltese drug legislation in 2006 and 2007 respectively [310, 311], the use of these substances appears to have declined since then. Moreover, the increase in the mean MDMA content of ecstasy tablets seized in Malta in 2011 (64.6 mg in 2011 from 57.9 mg in 2010) and in the UK in 2010 (43.5 mg in 2009 to 49.0 mg in 2010 [339]) could be an indication that the MDMA is recovering both in Malta and the UK as have been suggested by the UN 2012 World Drug Report [309].

A few cocaine powders contained the adulterant lignocaine, a local anesthetic and antiarrhythmic drug. However, more than half of the powders contained the adulterant phenacetin, an analgesic which was banned in 1983 in the US by the Food and Drug Administration because of its carcinogenicity and because it could cause kidney failure [41, 312]. Other psychoactive substances found in the powders included mephedrone and amphetamine (both stimulants), lignocaine and ketamine, a substance which is used as an anesthetic both in human and veterinary medicine. Ketamine, which

has spread in many parts of the world for its recreational use [313], has a stimulant effect at low doses and a psychedelic effect at higher doses [314]. Some powders contained no illegal or pharmaceutical substances, while others contained caffeine or paracetamol. The use of unidentified powders and the occurrence of powders containing paracetamol and caffeine in wraps could possibly have been done in order to mimic illicit drugs [306]. The presence of heroin (opioid analgesic), found in some brown powder, was not expected because these dance events are not usually associated with depressants; however, similar findings have been reported in the literature [41].

The finding in these seizures of a small amount (3 mL) of transparent liquid containing GBL was also important. The substance GBL, which is a prodrug to gamma-hydroxybutyric acid (liquid ecstasy), could be used in committing drug-facilitated sexual assaults, [315, 316]. Prescription tablets (1.6%) which included dihydrocodeine (a semi-synthetic opioid analgesic); counterfeit Viagra ® (used to treat erectile dysfunction) and promethazine (an antihistamine) were also detected in these seizures.

It was also noted from the seizures the subtle change in the drug use at major dance events where new substances such as ‘legal highs’ or ‘herbal highs’ started to emerge. These included mephedrone, a synthetic stimulant detected in Malta in 2010, and the cannabinoid antagonist JWH-018, detected for the first time in Malta in 2011.

The different types of illegal drugs found in these seizures, also reflected the occasional polydrug use by partygoers at major EDM events in Malta. It has been reported that polydrug use (use of two or more drugs at the same time or the active use of more than one drug by users [317]), frequently occur during recreational activities [318]. Although users tend to prefer certain psychoactive substances in combinations, it does not solely depend on personal preferences, but also on the availability, the price, the perceived quality, legality and fashion of the drugs [242, 318]. Moreover, drug users frequently change to other drugs if the necessity arises, such as when piperazine containing ‘ecstasy’

tablets and cocaine replaced MDMA tablets when the availability of this substance was low [309, 318].

Recreational polydrug users could also use a combination of drugs to get targeted effects such as the use of 'ecstasy' tablets or cocaine to increase endurance during dancing, and cannabis to relieve the unpleasant come-down effects, such as dysphoria and depression [65, 309, 319]. These combinations were noticed in the examined seizures where cannabis items were found together with 'ecstasy' tablets, or cocaine. The UN World Drug Report, 2012 claimed that during polydrug use the sequential use of drugs is most common [309]. However, sometimes MDMA tablets are also taken with cocaine and although there is evidence that the pleasurable effects of MDMA is increased when taken with cocaine, the mixture is claimed to be mostly dangerous because of the neurotoxic action of MDMA [320]. For this reason, while polydrug use could enhance the intended effects for the user, it could also cause serious health consequences due to the possible risk of increasing the adverse health effects of the drugs used [309].

6b Assessing the behaviour of participants at a major EDM event in Malta

In the last decade, clandestinely organised rave events have become more main stream, and well organised commercialised party culture [321]. 'Ecstasy' users began to consume other substances such as alcohol, cannabis, cocaine and amphetamines [112].

6b.1 Introduction

In Malta, as with many other countries, the organization and participation at major EDM events have always been influenced by changes happening on the European continent, especially in the UK [49]. England, which is considered to be a key city for EDM and rave culture [50], was and still is one of the main holiday destinations for many Maltese [322]. This influence has extended to the large outdoor summer music festivals which

are being organized around Europe, even in Malta, and which have also been linked with illegal drug use, especially 'ecstasy'. While it is generally acknowledged that illicit substance use, such as the use of MDMA, is common at EDM parties [41] this is largely hidden making it difficult to obtain objective data on the type of illicit substances being consumed by the interviewees. Previous research conducted at EDM events, have focused primarily on demographic information of drug users, their method of drug use and the supply and availability of drugs at such events. Yet there are still important gaps on the understanding of the attendee behaviour at these events [323].

The commercial nature of major EDM events is a key limiting factor, making the events largely impenetrable for research studies to obtain this type of data because of the fear of bad publicity by the organisers [324]. However, the differences between the late 1990s raves and the new commercial outdoor major EDM events require a better understanding of the behaviour of attendees before, during and after the event. Young people like to socialize and going out had become an important part of their lives [218]. Thousands of young Maltese (66.9%, n = 59,254) had attended to nightlife venues during 2007, with nearly one fifth of these (16.2%, n = 14,362) claiming to like EDM [325]. It is claimed that illicit drugs are normally found at these venues and that the prevalence use of these drugs at these nightlife setting is much higher [218]. Thus a study was conducted to investigate the behaviour at a major EDM event in Malta and the use of both licit and illicit substances.

This research, which was self-report based, sought to investigate details about:

- the way participants prepared for the party;
- their activity during the party;
- their activities in the after party phase;
- the use of licit substances, such as alcohol and nicotine;
- the possible use of EOCD;
- the after effects experienced by those interviewed following the party.

6b.2 Methodology

The study was approved by the Foundation for Social Welfare Services, Sedqa Ethical Committee on the 11th of August 2010 (see Appendix 3). Sedqa is Malta's National Agency Against Dependencies. Permission was also granted to use the Sedqa logo on the questionnaire.

6b.2.1 Research setting

The study was conducted during Malta's peak tourism season in August 2010. The "commercial" mainstream EDM event was organised by partnership between event promoters and leisure companies. It was held at an outdoor club located in the centre of the island, in large open space around 5 km from the closest inhabited areas. The club, which is surrounded by trees, has one of Malta's largest dance floors and has a capacity of over four thousand people. The club owners granted the authors permission for the study and facilitated the access for the researchers to attend the event.

6b.2.2 Procedure for data collection and analyses

The questionnaires were administered by ten interviewers, who had previously undergone training regarding the recruitment and data collection (Appendices 4 and 5). The questionnaire was semi-structured and available in Maltese and English. The questionnaire (see Appendix 6) was composed of five sections which sought information on:

- demographic information;
- in-party behaviour;
- EOCD drug consumption;
- use of ecstasy;
- post-party behaviour.

1. The ***demographic*** section, which sought information on the age, nationality, education status and employment, helped to determine and describe the interviewed population.

2. Interviewees were asked about their ***behaviour*** during the event itself in order to determine if their actions during the event were predetermined from the illegal drug they intended to or had consumed. In addition the questions were set such that the answers also tried to discriminate between those interviewees who used only 'ecstasy' and those who used 'ecstasy' and / or other illegal drugs.

3. The section on ***drug consumption*** provided information about patterns of drug use in the interviewee. This section also includes the Alcohol Use Disorder Identification Test (Audit-C) [326].

4. The section on the ***use of ecstasy*** contained questions which dealt with the possible use or otherwise of 'ecstasy' by interviewees at the EDM event.

The information obtained from Sections 3 and 4 assisted in determining the quantity and type of illegal drugs consumed at these events and also provide some information on the dimensions in the local Maltese context.

5. The section on ***post-party behaviour*** provided information on the way interviewees sought to minimise the negative effects of any drugs they may have consumed. They were asked about what they did if they became unwell at the event, whether they took fruit or vitamins after the event and if they did anything to help them sleep after the event. In addition this section sought to determine if there were any similarities and differences between those who used only 'ecstasy' and those who used 'ecstasy' and / or other illegal drugs.

The coded and fully anonymised questionnaires were analysed as described in Chapter 2, Section 2.4.2.

6b.3 Results

Demographic data

A total of 58 subjects took part. There were more male interviewees (70.7%, n = 41) than females (29.3%, n = 17). Their mean age was 24.98 years (SD 6.11, modes 19, 20, 22 and 24, range 16 - 43 years). Nearly all the interviewees (96.6%, n = 56) were Caucasian (white), with a large majority (60.3%, n = 35) being Maltese. About a third (36.2%, n = 21) of those interviewed were tourists coming mainly from European countries. Almost all of the interviewees (94.8%, n = 55) listed upper secondary and tertiary level as their highest level of completed education. The majority of the interviewees (67.2%, n = 39) were fully employed and most (63.8%, n = 37) still lived with their parents.

Party behaviour

While over half (59.7%, n = 37) of the partygoers attended these parties with friends, in groups of 4 to 10 people, 27 of the interviewees (46.6%) stated that they informed their friends that they were going to the event. Moreover, 26 interviewees (44.8%) reported that they go to a party every week. Before attending the event, two thirds of the interviewees (67.2%, n = 39) stated that they usually had a good meal or vitamins or minerals. Most (81.1%, n = 47) of those interviewed attended the event for between 4 to 6 hours, with the majority 82.8%, (n = 48) of those attending spending between 40-80% of the time dancing. The mean money spent during such an event, according to interviewees, was € 59.5 euros (SD 51.5, range € 0 - 200).

Alcohol and tobacco use

All but one (98.3%, n = 57) of the interviewees were found to have consumed alcohol at least once, during the year and in the month before the interview took place. Most (96.55%, n = 56) of the interviewees stated that they had consumed alcohol during the event itself. The mean age for the first time consumption of alcohol among interviewees

was 15.44 years (SD 2.5, range 11 – 24 years). The interviewees were found to consume alcohol on a mean of 11 days during a month (SD 7.62).

More than half (62.1%, n = 36) stated that they drank ≥ 8 units of alcohol. The mean number of units drank at a typical party was 11.05 (SD 35.37, range 0 – 30 units). The Alcohol Use Disorder Identification Test (Audit-C) was used as a diagnostic tool to screen for alcohol use disorders in the subjects Table 6b.1). The majority (87.7%) of the

Table 6b.1 Results from "Alcohol Use Disorder Identification Test" (Audit-C). The table shows the percentage results of the frequency alcohol consumption of the interviewed participants at the EDM event.

Audit-C	**Scoring system**				
	0	**1**	**2**	**3**	**4**
Frequency of drinking	**Never**	**Monthly or less**	**2-4 times per month**	**2-3 times per week**	**4+ times per week**
Number of subjects responded	1	4	20	15	17
Quantity of units of alcohol consumed on a typical day	**1-2**	**3-4**	**5-6**	**7-9**	**10+**
Number of subjects responded	4	5	18	11	19
Alcohol consumption on single occasion during the last year: Female ≥ 6 units and Male ≥ 8 units	**Never**	**Less than monthly**	**Monthly**	**Weekly**	**Daily or almost daily**
Number of subjects responded	7	9	8	29	4
Scores	**0-7**		**8 (cutoff)**	**9-12**	
%	**37.5, n = 21**		**16.1, n = 9**	**46.4, n = 26**	

interviewees confirmed consumption ≥ 6 units or ≥ 8 units on a single occasion during the last year. While the majority (84.2%, n = 48) reported that they would consume 5 or more units of alcohol when they were drinking, more than half (56.1%, n = 32) of the

interviewees stated that they drank ≥ 2 to 4 alcoholic drinks per week (Table 6b.1). The Audit-C showed that nine (16.1%) of the interviewees were a score of 8, an indication of hazardous and harmful alcohol use [327]. As shown in Figure 6b.1 it can be seen that most subjects (62.5%) scored between 8-12 on the scale, while 16.1% (n = 9, males scored 4 to 5 points, and females scored 3 to 5 points) consumed alcohol with moderation (Figure 6b.1). More than one third (39.7%, n = 23) of the interviewees stated that they did not drink any non-alcoholic drinks.

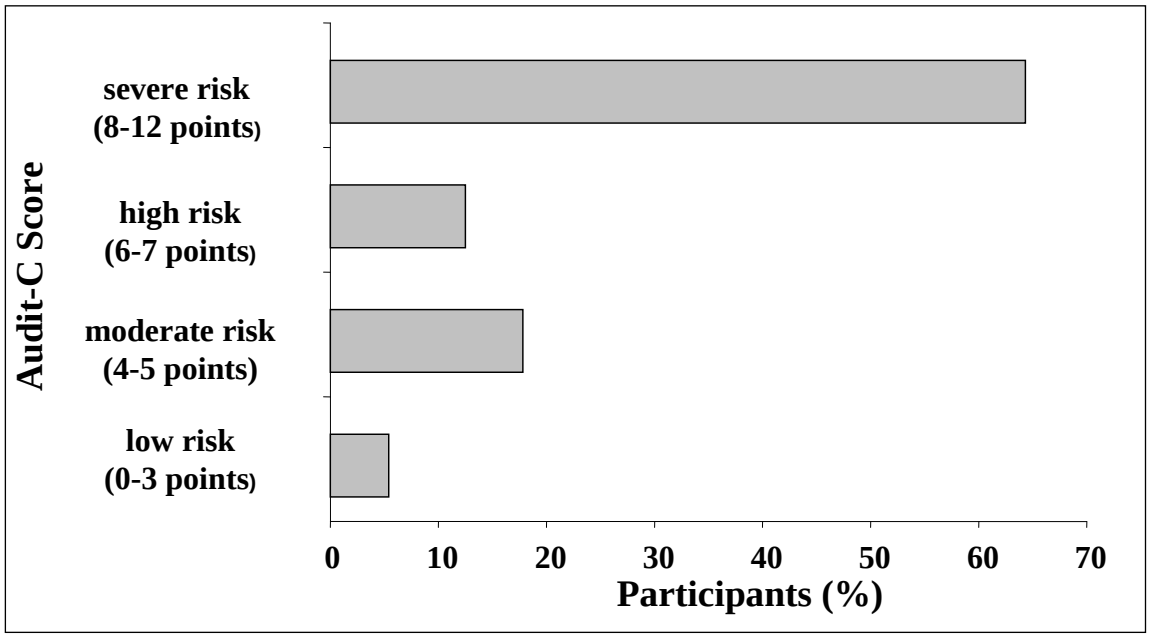


Figure 6b.1 Graph showing the percentage results of the Audit-C Score. Men who score 4 or women who score 3 or higher, drink above recommended limits and are at increased risk of harm (56 replies).

Most of the interviewees (75.9%, n = 44) were found to have ever used tobacco, in the form of cigarettes. The mean age for first use of tobacco for those who answered (58.8%, n = 20/34) was 15.62 years (SD 2.15, range 12 - 21 years). More than half stated that they had smoked on the day of study (60.3%).

Illicit substance use

Just over half of the subjects (55.2%, n = 2) reported 'ever-use' of illicit drugs.

Cannabis (such as resin, grass, oil and skunk) were found to be the most popular drugs (87.5%, n = 28) among those who claimed to have 'ever-used' illicit drugs (Figure 6b.2). The use of cannabis was also the most common illicit drug used among EOCD users during the year and month of the study (46.9%, n = 15 and 37.5%, n = 12 respectively). Half of the subjects (50%, n = 16) who stated 'ever-used' of illicit substances had used 'ecstasy' (tablet and / or powder) and 15 (46.9%) interviewees had used cocaine (powder and / or crack) (Figure 6b.2). The use of these two illicit drugs by the EOCD users during the year and month of the study was: for the 'ecstasy' items 37.5% for the tablets (n =12) and 21.9% for the powder (n = 7) respectively and for the cocaine items 15.6% for the powder (n = 5) and 3.1% for the crack (n = 1) respectively. Other reported

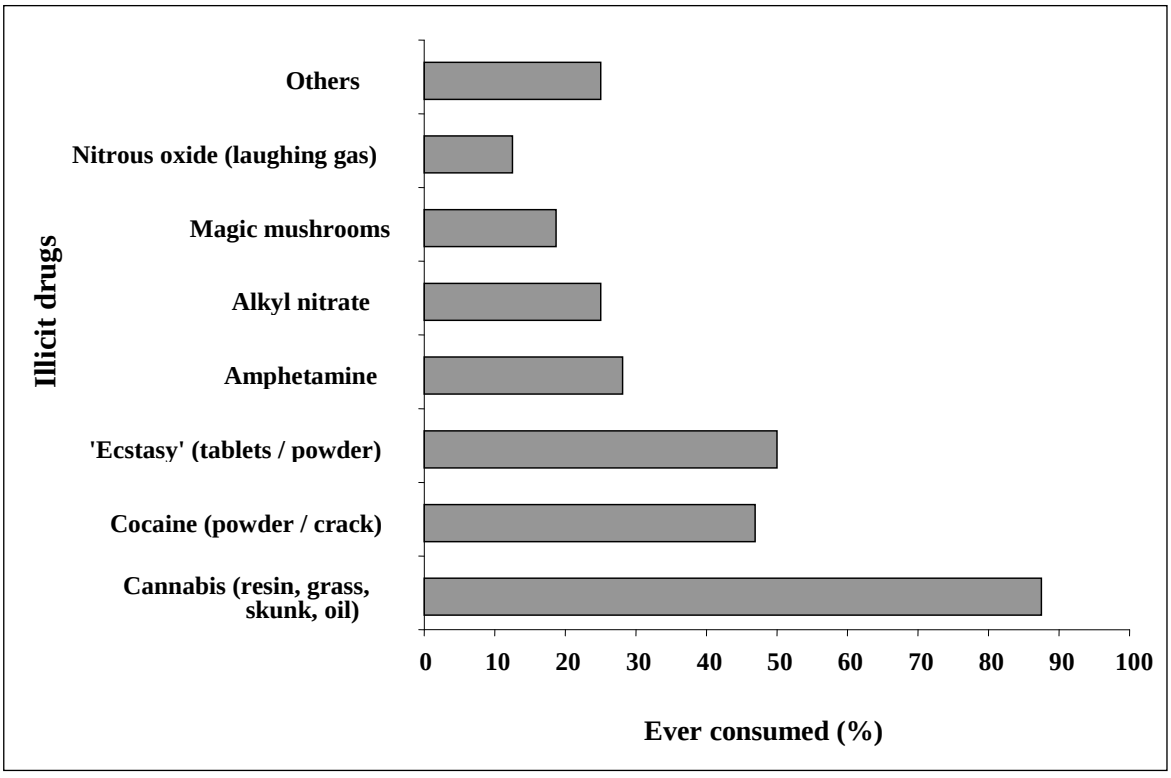


Figure 6b.2 Percentage of illicit drugs that were consumed by interviewees some time in their lives.

'ever used' EOCD (Figure 6b.2) by the interviewees included amphetamine (28.1%, n = 9), alkyl nitrate (25.0%, n = 8) magic mushrooms (18.8%, n = 6) and nitrous oxide

(12.5%, n = 4). Additionally ketamine, LSD, salvia devanorum, MDA, MA, mephedrone, and gamma-hydroxybutyric acid were reported as 'ever-used' as were heroin and opium.

The mean age of 'first-time' use of illicit drugs among subjects was 19.69 years (SD 4.03, range 13 – 24 years). The mean age for the 'first-time' of 'ecstasy' (tablets and / or powder) was 20.95 (SD 4.09, range 14 – 33 years), for cannabis mean 17.24 years (SD 2.73, range 13 – 25 years) and for cocaine (powder and / or crack) mean 20.58 years, (SD 2.71, range 17 – 25 years).

Among the interviewees who 'ever-used' illicit drugs, 40.6% (n = 13/32) reported using them in the month prior to the study. The majority of the interviewees who attended the EDM event did not report any use of EOCD. Only four interviewees stated that they had smoked cannabis on the day of the event. No significant difference ($p > 0.05$, one-way ANOVA) in the party behavior (i.e preparing, informing friends, before party, etc. see Appendix 6) of the interviewees was detected between those who claimed to have never used (43.1%, n = 25) and those who ever used illicit drugs (55.2%, n = 32). Moreover, there was no significant difference ($p > 0.05$, one-way ANOVA) between those interviewees who claimed to have never used and 'ever-used' EOCD, irrespective of gender, for alcohol consumption.

Use of 'ecstasy'

Nine subjects who had 'ever-used' 'ecstasy' tablets (24.1%, n = 14), reported that on average they consumed between one to four tablets for each event and four of these interviewees added that they took 'ecstasy' for only one day. Eight of the interviewees felt that it was important that 'ecstasy' tablets contained MDMA and a further eight would test their tablets if they could for curiosity and / or for health concerns. Only seven of the 58 subjects had used 'ecstasy' tablets during the month before the study.

Although on the night of study none had used 'ecstasy' according to self-reports the prices quoted for 'ecstasy' tablets and powder, ranged between € 2.5 to 10 per tablet, and between € 20 to 80 per gram of MDMA powder. Out of the sixteen interviewees who declared ever used 'ecstasy' (tablets and powder), twelve stated that they did not prefer the powder. Those that did prefer the powder did so because of its supposedly better quality. Interviewees (n = 4) took other drugs with 'ecstasy' tablets such as Viagra®, LSD, ketamine and mushrooms. Alcohol was found to be the most common licit substance (93.8%, n = 15), followed by tobacco (81.3%, n = 13) and the illicit cannabis-items (75%, n = 12) that were mostly taken by the ever used 'ecstasy' partygoers.

Of the 16 subjects who had previously used ecstasy the most common side-effects reported was excessive sweating (13/16), thirst / dehydration (10/16), numbness / tingling (6/16), feeling dizzy (6/16) and on occasion unable to pass urine (5/16). Others stated that on occasion they vomited or felt nauseated (3/16) and had sexual problems (2/16).

Post-party behaviour

Asked to record how they felt at the EDM event nearly a third (34.5% n = 20) declared sometimes feeling unwell. There were significance differences between interviewees who 'ever-used' illicit drugs and those who did not report drugs. Those who 'ever-used' slept longer (8 h vs 5 h, $p < 0.05$, one-way ANOVA). However, there was no difference ($p > 0.05$, one-way ANOVA) in alcohol drinking.

6b.4 Discussion

This study provided some insight and understanding into the context of attendee's behaviour at a major EDM event in Malta. The average event-goer was found to be about 25 years old, well educated, most probably employed and still living with parents (as is the norm for many young persons in Malta [328].

6b.4.1 Alcohol and tobacco

Alcohol was found to be the most commonly used licit substance at this EDM event. More than half (52.6%, n = 30) of those that reported alcohol use on a typical day would consume more than half of the national average weekly units of alcohol for those aged between 18-34 years (12.7 units for those aged 18-24 years and 13.3 units for those aged 25-34 years [325]. Of those interviewed 75.9% were found to be high to severe risk alcohol drinkers (scored 6-12, Audit C). These results were found to be similar to results obtained in the Scotland study [324]. Most of the interviewees were found to be smokers and more than half (60.3%) were current smokers.

6b.4.2 Illicit substances used

The majority of the interviewees did not report any use of illicit drugs on the night at the EDM event. However, respondents in this study who once used or were using illicit substances were more likely to use psychedelic drugs. Cannabis items were the most preferred illicit drugs that were used by users in this study. Also, a fair number (36.21%, n = 21) of interviewees in this study stated that they had experimented with stimulants, such as 'ecstasy', cocaine and amphetamine, among others. 'Ecstasy' tablets were preferred to the powder; however, those that did prefer the powder did so because of the perceived better quality. However, although most of the users of 'ecstasy' tablets were concerned about the contents of tablets they did not bother to test them.

Other drugs reported to be 'ever-used' included the psychedelics magic mushroom and LSD, and the stimulants amphetamine, MA, mephedrone and herbal ecstasy. The use of anesthetic drugs, such as gamma-hydroxybutyric acid and ketamine, did not seem popular with the drug users at such events because only a very small number of participants stated that they had at some time used these substances. Illegal depressants, such as heroin and opium which are not normally found at EDM events, were also reported to have been used at least once in a life-time by a very small number of participants. However, this is not surprising because the very nature of the effect of these

substances would impair dancing ability. It is also worth noting that most of the licit and illicit substances mentioned by respondents of this study were similar to the drugs seized by the Malta Police Drug Squad over a six year period, 2006 - 2011, (Chapter 6, Section 6a.3.1) from 18 large open-air EDM events in Malta.

6b.4.3 Party behaviour of attendees

The party behaviour of interviewees attending this open-air EDM event was also examined. The interviewees were asked about how often they attend such events, with whom they went to the party, what they did before a party, the length of time they partied in one go, the time spent dancing, the money spent on a typical night and the type of drinks they consumed at the event.

Going through the behaviour results a typical attendee to this major EDM Malta event would have taken a good night sleep and would have informed his friends before going to the event. The young adult would normally have attended the event with 4 or more friends and partied for 4 to 6 hours with half of the time spent dancing. Becoming unwell at EDM event is not uncommon even if illicit drugs are not used. While the hot and crowded atmosphere could be one of the reasons for this, the most probable cause would have been the high alcohol consumption which was not uncommon at the studied event. After the event, if not going to an after party, the event-goer would normally sleep for less than 8 hours, without taking anything to sleep.

6b.4.4 Overall findings

The rate for lifetime use of alcohol (98.3%) followed by cannabis (48.3%), 'ecstasy' (27.6%) and cocaine (25.9%), which were found to be relatively high in this study when compared to the general population, were found to be similar to a study conducted among Swiss partygoers [305].

The high use of alcohol at this specific Malta EDM event was found to be similar with

findings from other studies that were conducted on EDM event-goers in other countries including Belgium, UK and Australia [218, 242, 329]. In these studies interviewees claimed that at these events alcohol can be used as a substitute for the most commonly used EOCD, such as cannabis, ecstasy and cocaine, but no substance was able to replace alcohol [242].

This further supported the idea that those who are poly EOCD users are primarily alcohol users [218, 242, 329]. Similar findings were reported by Riley et al. [219] and then by Measham [330, 331] who found that there was a decline in the use of cocaine and 'ecstasy' tablets and increase in alcohol consumption in EDM events [219, 330, 331].

Measham, in 2006, claimed that the increase in alcohol consumption and the decrease in illicit EOCD use were caused by the merger of the "legal and illicit psychoactive markets, with normalization of recreational drug use" [331]. Similarly Martinus et al. [324] again maintained that alcohol was the main substance used, whilst illicit drug use was minority behaviour [324]. Whereas in the late 1990s, the EDM scene was associated with club drugs ('ecstasy', LSD, amphetamine and cannabis) and alcohol popularity was then low; in the last decade the use of alcohol had greatly increased in the EDM event scene [324]. In addition, similar to other studies, interviewees in the Malta study were also found to be predominantly current tobacco smokers [329, 332].

It was also evident that the three most common illicit drugs 'ever-used' were (in order most frequently mentioned): cannabis, 'ecstasy' and cocaine. This could be an indication that cannabis, 'ecstasy' and cocaine are the most popular EDM drugs among Maltese partygoers similar to their counterparts in the UK [2, 333]. The drug types did not differ significantly between 'ever-used', used this year and last month. Although on the day of the Malta event very few attendees stated to have used an illicit drug, probably due to the presence of plain-clothes policemen at the party, most of the subjects did not avoid from alcohol consumption, being legal. This could have caused problems if the partygoer was a polydrug user. The use of illicit drugs together with alcohol consumption is not

uncommon in these types of events [363, 334]. Polydrug use may be due to a need by the user for drugs serving different purposes such as stimulants being used in the early part of the event in order to increase energy levels to dance, whilst the use of alcohol and cannabis later on in the event would serve to decrease agitation and sleeplessness [333].

This study also provided a distinct sequence for the age of first use for the most popular EOCD. Most users began to use alcohol and tobacco at a mean age of about 15 years, followed by cannabis, for those who used illicit drugs, at a mean age of about 19 years. 'Ecstasy' and cocaine products, when consumed, were first taken at a mean age of 20 years. Although the pattern of first use of these substances was comparable to a similar study conducted in 2001 in three European cities, Amsterdam, Hanover and Vienna (14 years for alcohol and tobacco, 15 years for cannabis, 17 and 18 years for 'ecstasy' and cocaine respectively) the ages of onset were found to be higher in the Malta [332].

Of the 16 people that reported ever using ecstasy it was also established that one to four 'ecstasy' tablets were taken by users at a party whenever they used this drug. This finding was similar to other previously published studies [2, 335, 336]. Presumably the number of 'ecstasy' tablets taken during a night out would be dependent on the duration of the party, personal preferences and the quality of the 'ecstasy' tablets with regards to the amount and the type of active ingredient present.

As already stated in the introduction to this part of the chapter, the use of stimulants in EDM events, are seldom the centre of recreational activities [337]. In effect the majority of the interviewees who attended the Malta EDM event did not report any use of EOCD. However, the few that did use EOCD did so to enhance some other activities such as social interaction [337]. Contrary to what was expected the behaviour at the event and the post-party period of those who ever used or continued to use EOCD was not different from those who never used these illicit drugs. However, the most important finding of this Malta study was the high dangerous use (score 8-12, Audit-C) of alcohol**.**

6b.4.5 Limitations of the study

This study, as with most research studies, has some methodological limitations which need to be acknowledged. The questionnaire which had to be kept short, since it is difficult to keep the interviewees attention for a long time during an EDM event, did not delve deep enough into the behavior and substance use of the clubbers. The findings should be interpreted with some caution since the information given by the interviewees could have been subject to errors in recollection.

In addition, the presence of plain-clothes policemen during the event might have affected the interviewees' responses, causing them to under-report their EOCD use. As with all studies involving questionnaires to possible EOCD users, the use of the mentioned drugs cannot be taken confirmed since the 'ecstasy' available may not have contained MDMA but other substances.

This study however, as far as can be found, was the first Maltese study which sought to investigate the behaviour of EDM event attendees and use of EOCD at major EDM events and a number of interviewees were willing to talk while at the event. The results from this study could be used to formulate strategies and education campaigns as part of primary prevention activities by the relevant authorities.

Chapter 7

CONCLUSION

As discussed in Chapter 1, this thesis had tried to address of how physical characterisation of ecstasy tablets could be used to establish links and contrast and compare tablets found in specific batches for use for forensic drug intelligence purposes. Similarity in the physical features of tablets normally indicates that the tablets were from the same batch and that the same tabletting machines and punches were used [136], whilst difference in the physical features of tablets is usually indicative that these were from different batches. It was for this purpose that the physical characterisation of batches of 'ecstasy' tablets, which included both the visual features (shape, colour, logo and colour) and the measurable features (mass, diameter, thickness, hardness, friability and disintegration rates) were investigated. However, the physical features of tablets are dependent on the storage conditions of the batches after manufacture. Thus stability studies focusing on the photostability, temperature and RH, were conducted to investigate possible changes in the physical features of tablets and to propose an ideal storage conditions for the 'ecstasy' tablets while held at the forensic laboratory. This was to ensure that the physical characteristics of the tablets in the batches remain stable throughout an investigation.

It is sometimes argued that the physical features of tablets are not sufficient enough to link or differentiate between batches of 'ecstasy' tablets [212] and chemical characterisation is recommended. Thus chemical characterisation of 'ecstasy' tablets from seized different batches was undertaken using in the process experiments to determine the psychoactive substances both qualitatively and quantitatively of the tablets, determining the isomeric content ratio and impurity profiles of MDMA-only tablets, determining the major excipients and elemental profiles of the tablets from the batches.

The chemical characterisation, which complemented the physical characterisation, further helped link or differentiate between the examined seized batches of 'ecstasy' tablets.

This research has also tried to investigate the use of 'ecstasy' tablets at EDM parties, for this purpose two studies were conducted. The first study involved the analyses of substances and tablets which were confiscated by the police during 18 "commercial" EDM parties in Malta over a six year period from 2006 - 2011. The second study, which was called the "Malta study", investigated the behavior of participants and their use of both licit and illicit substance at a "commercial" EDM event held during the summer of 2010.

The present research utilised a unique national collection of 'ecstasy' tablets which were seized from traffickers by legal authorities and 'ecstasy' tablets and substances which were confiscated by the police during EDM parties, in Malta. The 'ecstasy' tablets from traffickers consisted of 45 batches (total 98,653 tablets) which were seized over a five year period (2006 - 2011). From the EDM parties a total of 172 seizures, suspected to be drugs of abuse and containing a total of 75 tablets mostly 'ecstasy' tablets (94.7%, n = 71), were confiscated by the police during the commercial" EDM parties, over a six year period (2006 - 2011).

During the period of study the European 'ecstasy' market has changed and this might have affected the small illicit drug market in Malta. Hence, it will be demonstrated whether the Maltese batches of 'ecstasy' tablets that were used in this study also reflected the changes that occurred in the European 'ecstasy' market during the five year period (2006 – 2011). It will also be discussed whether the data and results from this thesis can be extrapolated to the European-wide scenario and be used in the forensic analyses of ecstasy found across Europe.

7.1 The 'Ecstasy' Drug Market in Malta and Europe, 2005 - 2011

In 2005 in Europe 'ecstasy' tablets that were supposed to contain MDMA started to

contain other psychoactive substances creating instability in the 'ecstasy' market [309]. Part of the reason for the use of other psychotropic drugs in 'ecstasy' tablets was attributed to the lack in the availability of the precursor chemical 3,4-MDP2P which caused a decrease in the production of MDMA [309, 338]. The decrease in the availability of the chemical 3,4-MDP2P was credited to the successful investigations by the Dutch and Belgian into Chinese organized crime [309, 338]. The decline in the production of MDMA also caused a reduction in the content of MDMA in 'ecstasy' tablets [309]. Moreover, by 2006, the number of 'ecstasy' tablets seized in Europe started to decline, even more in 2008 [1, 213] until the drastic decline in 2009 (12.7 million tablets in 2008 to 1.9 million in 2009 seized tablets, EU and Norway) [339, 340]. Nevertheless, by mid-2010 Europol reported a resurgence of 'ecstasy' tablets in Europe [309]. The revival of the 'ecstasy' tablets was confirmed when three million tablets were seized in Europe in 2010 [341]. However, the resurgence in 'ecstasy' tablets seems to have occurred without the re-emergence of the primary MDMA precursor 3,4-MDP2P, indicating that clandestine chemists were using new precursors, or pre-precursors, to manufacture MDMA [309].

The European 'ecstasy' market also affected the UK illicit drug market. During the same period, 2006 - 2009, the number of 'ecstasy' tablets that were seized in the UK had decreased drastically (from ~ 6.7 million in 2006 to 171 thousand 'ecstasy' tablets in 2009 [342]). Similar to what had happened in Europe in the UK by the mid-2008 'ecstasy' tablets containing piperazines outnumbered 'ecstasy' tablets containing MDMA, implying that other psychoactive substances were being used instead of MDMA. However, when in 2009 the piperazines became controlled substances in the UK, the number of tablets containing piperazines fell sharply [308]. Moreover, the resurgence of 'ecstasy' tablets that was noticed in Europe in 2010, was also witnessed in the UK (357 thousand 'ecstasy' tablets seized in 2010 [308]). By 2010, the purity of 'ecstasy' tablets for the substance MDMA had increased, further confirming the revival of the 'ecstasy' market in the UK [309].

In Malta, no 'ecstasy' laboratories have ever been found, and police investigations have determined that most of the 'ecstasy' tablets imported to the Island, come from European mainland, especially from the Netherlands (N. Harrison, 2012, personal communication, Assistant Commissioner, Malta Police, interview, 12 June). Thus any changes which occurred in the European 'ecstasy' market during the study period 2006 - 2011 would have been expected to affect the Maltese illicit drug market.

However the study came up with some key differences. Unlike the situation in Europe, where there was a decrease in the number of 'ecstasy' tablets seized, in Malta between 2006 and 2010 the number of 'ecstasy' tablets seized, which contained MDMA, was fairly stable averaging 20,376 tablets annually (N. Harrison, 2012, personal communication, Assistant Commissioner, Malta Police, interview, 12 June). However, as happened in the European market, 'ecstasy' tablets containing other psychoactive substances, also started to be seen in Malta during the period of the research study. The highest number of 'ecstasy' tablets, a total of 67,182, was seized in Malta in 2006. One of the 2006 seizures, which contained 50,703 'ecstasy' looking tablets, contained the piperazine substance mCPP instead of MDMA. Moreover, in that period mCPP was not a scheduled substance in Europe including Malta and this may have accounted for this unusually large seizure. In 2006, small amounts (< 150 tablets) of 'ecstasy' tablets containing another piperazine substance, BZP, were also seized.

Contrary to what was happening in Europe in 2010 with regards to the resurgence of 'ecstasy' tablets, in Malta the opposite occurred. In 2011 and 2012 there was a drastic decrease in the numbers of 'ecstasy' tablets seized (2,143 and 1080 tablets respectively, N. Harrison, 2012, personal communication, Assistant Commissioner, Malta Police, interview, 12 June]). The decrease in the seized number of 'ecstasy' tablets was probably due to a new psychoactive drug, 4-methylmethcathinone **(**mephedrone) in powder form, which had appeared by the mid-2010 on the Maltese illegal market. By the end of 2010, this new psychoactive substance, 4-methylmethcathinone, was the most common drug seized at EDM parties and was probably the cause for the displacement of 'ecstasy'

tablets from these parties. So overall there were some differences between the European and the small Maltese 'ecstasy' drug markets.

7.2 'Ecstasy' Tablets: Physical Characterisation, Stability and Chemical Profiling

As already described the prime objectives of this thesis was to establish methods for physical and chemical characterisation of 'ecstasy' tablets which could be used to establish links between batches of tablets in a forensic drug intelligence perspective. This study has demonstrated that the methods used and developed in Chapter 2, "Materials and Methods", have a potential in developing forensic science intelligence for the supply and manufacture of 'ecstasy' tablets. Another prime objective of this research was the study which examined the effects of light, temperature and humidity on the physical measurable features of the tablets. This study might influence the conclusions that could be drawn from using physical characteristics to link or discriminate between batches. The forensic scientists might need to take into account the possible changes in physical characteristics of tablets that might have taken place before seizure such that two batches from the same origin but which have been subjected to different environmental conditions during transport / handling may not give a match through physical characterisation.

7.2.1 'Ecstasy' tablets: Physical characteristics

The batches of 'ecstasy' tablets that were examined in this study were mainly imported from the Netherlands, the country mostly associated with large 'ecstasy' production (N. Harrison, personal communication, Assistant Commissioner, Malta Police, interview, 12 June). In the Netherlands, 'ecstasy' tablets are normally manufactured by high capacity tabletting machines, producing between 15,000 to 60,000 tablets per hour [338].

The results from the physical characterisation concluded that similar tablets from the same and different batches having similar logo, shape and colour had very similar

measurable physical features, such as mass, diameter and thickness, thus indicating the possibility that the tablets were produced by the same tabletting machine and punches [139]. This part of the study showed that the visual features logo, shape, breakline and friability were found to be very reliable features to be used to characterise and discriminate between batches of 'ecstasy' tablet, however the measurable features mass, diameter, thickness were found to be the most discriminating features. It was also determined from this study that the 'ecstasy' tablets that were seized on different occasions did not always have different physical features thus the set ***hypothesis (1)*** that:

> ***The physical state of different batches of 'ecstasy' tablets seized on different occasions in Malta during 2006 – 2011 was significantly different from each other.***

was partially satisfied because 14 batches confiscated during 3 out of the 30 seizures were found to have similar features.

It was also concluded from this part of this study that there was good compliance for the pharmacopeial and pharmaceutical criteria for the individual physical measurable features of the examined batches of 'ecstasy' tablets. This indicated the good expertise available to the illicit chemists producing these tablets and to the use of more professional tabletting machines found in the illicit 'ecstasy' market [338].

7.2.2 'Ecstasy tablets': Stability of colour and measurable physical features

If the physical features of 'ecstasy' tablets are to be used for intelligence purposes these characteristics should be protected during the storage of tablets at the laboratory. A study was conducted to try and determine how the light, both visible and UV, would affect the colour of tablets. Two other studies were conducted to determine how different temperatures and RH would effects the measurable features (mass, diameter, thickness, friability, hardness and disintegration) of the tablets. The stress tests used to determine changes in the colour and the measurable features of 'ecstasy' tablets were adopted from International Conference of Harmonisation methods.

In the literature the general consensus is that colour and colour variation of 'ecstasy' tablets are not reliable characteristics for discrimination because interpretation is "highly operator dependent" [181]. No studies have been published which characterise 'ecstasy' tablets by their colour and colour variation using instrumental evaluation. However, instrumental evaluation methods using reflectance spectrometry have been used to evaluate the stability of dyes in pharmaceutical tablets [230]. In this research study 'ecstasy' tablets were subjected to photostability stress conditions to investigate change in the colour of the tablets, including white tablets, using reflectance spectrometry based on the CIE colour system.

This study confirmed that if the colour of 'ecstasy' tablets is evaluated by reflectance spectrometry it could be one of the physical features that could be used to characterise 'ecstasy' tablets. Portable spectrcolorimeters with data storage capacity, similar to the instrument used in this study, makes colour evaluation of seized batches of 'ecstasy' tablets easy and quick. The results indicated that the colour of the tablets changed under both visible and UV light. After 38 days obvious colour change was noted in the colour of orange tablets with the pisces logo (batch 14) but only slight to very slight changes in the colour was recorded for the blue and green tablets with the omega logo (batches 5 and 8) and white tablets with the euro logo (batch 11).

The two other stability studies, which involved the storage of 'ecstasy' tablets under different temperatures and RH, confirmed that changes do occur to the measurable features of tablets if stored at high 75% RH, and mostly when kept at a temperature of 40°C. The changes in the measurable physical features of 'ecstasy' tablets, such as the diameter, thickness and volume, which were caused by swelling of the tablets due to major excipients such as lactose, were found to be in accordance with results from experiments conducted on pharmaceutical tablets [253, 254]. The use of major excipients such as lactose permits the manufacture of tablets by direct compression, which is probably the method used for the manufacture for 'ecstasy' tablets [134], has the advantage of lower costs and tablet stability [253]. However, when tablets are produced

by direct compression using lactose as the major excipient the tablets tend to swell [253] when stored at high RH (≈ 75%), thus making it difficult to link batches of tablets if these have common origin. The hardness was also found to decrease more markedly when tablets were stored at 75% RH and 40ºC making it difficult and sometimes impossible to measure the tablets physical features. It was also determined from the stability study that the least change in the measurable features had occurred when tablets were stored at 33% RH and at temperatures between 15 and 35°C. Thus it is recommended that batches of 'ecstasy' should be stored at low RH (≈ 25%) and temperature (≈ 25 °C) and away from sunlight if the measurable features are to be used for intelligence purposes.

7.2.3 'Ecstasy' tablets: Organic and inorganic chemical profiling

All 'ecstasy' tablets taken from batches of major seizures were subjected to a general organic chemical profile to determine the major psychoactive substances present. More than half (66.7%, n = 30) of the analysed tablets were found to contain MDMA as their active ingredient. 'Ecstasy' tablets would not always contain MDMA as their major active ingredient and thus the tablets seized in Malta were found to contain other psychoactive substances such as DPIA, a psychoactive substance first detected in 'ecstasy' tablets in Malta in 2006 and reported to EMCDDA, mCPP, BZP, caffeine, the anabolic steroid methandrostenolone and tablets with no psychoactive substances. Thus ***hypothesis (2)*** which stated that:

> ***Seized 'ecstasy' tablets will contain MDMA as the predominant psychoactive substance.***

was not verified.

Although not much have been written about the use of the general organic chemical profiling of 'ecstasy' tablets for evidential and intelligence purposes [126], this should be used because it adds another discriminating factor that together with other features, such as physical characteristics, could help link or differentiate between batches of tablets.

Clearly there is no set recipe for the production of 'ecstasy' tablets and the drug content tends to vary between different batches of tablets. Hence, the drug content was another discriminator that was used to link or differentiate between batches of 'ecstasy' tablets. The European pharmacopeial method was used to determine the mean content of the psychoactive substance present in ten similar 'ecstasy' tablets that were taken from the same batch [221]. The results from these analyses have shown that in general the dose of the psychoactive substances between 'ecstasy' tablets from the same batch did not vary much (< 5% RSD). However, there was variation in the mean dose between different batches of tablets, normally having different physical features.

The batches containing MDMA tablets that were seized in Malta over the five year period (2006 – 2011) were also analysed to determine their enantiomeric composition. From the analyses it was determined that the batches contained tablets with a racemic mixtures of MDMA. Thus ***hypothesis (3)*** which stated that:

The enantiomeric ratio of MDMA tablets will be 50:50 in illicit tablets.

was verified.

Impurity profiling of seized MDMA tablets was another discriminator that was used to try and classify tablets into related batches. Impurities in the tablets are mainly generated by the precursors chemicals used in the synthesis of MDMA, impurities present in the starting materials, and by-products from side reactions produced during MDMA synthesis. Other generated impurities are caused by inadequate purification of MDMA after synthesis and by contaminants [190] that are carried over unchanged to the final synthesised product. The impurity profiling of MDMA tablets in this study focused mainly on identification of the precursors and the synthetic routes used. From the chemical profiling results it was determined that 3,4-MDP2P was the precursor mostly used and that two synthetic routes, the Leuckart or reductive amination reactions, were used to synthesise the MDMA found in the tablets.

The excipients of 'ecstasy' tablets were also investigated for their discriminating power. Major excipients were identified by means of FTIR transmittance spectroscopy, while the elemental profiling of some of the excipients present in 'ecstasy' tablets was done by SEM/EDX analyses. The compounds lactose and sorbitol were found to be the most common major excipients used in 'ecstasy' tablets, while elemental profiles of the excipients indicated the presence of magnesium stearate as the common lubricant, together with other possible glidants used in tablet production. Although the identification of the major excipients and the inorganic profiling methods used for the tablets were not found to be highly discriminating it could still be used with other characterisation and profiling methods to further help differentiate or link batches of 'ecstasy' tablets. From the chemical characterisation it was possible to link 14 batches of 'ecstasy' tablets containing MDMA as the major and only psychoactive substance. Thus ***hypothesis (4)*** which stated that:

> ***The chemical composition of different batches of 'ecstasy' tablets seized on different occasions in Malta during 2006 – 2011 will be significantly different from each other.***

was not verified.

In conclusion the two step process in the production of 'ecstasy' tablets, that of synthesis of the active ingredient/s and the production of tablets, provides many characteristics that could be used for linking or discriminating between batches of tablets. This study which mainly focused on the physical and chemical characteristics of 'ecstasy' tablets tried to provide methods and examples as to how these characteristics could be used for intelligence purposes. The measurable physical features mass, diameter and thickness together with the determination of the psychoactive substances and excipients, inorganic impurity profiling in the case of MDMA-only tablets and elemental profiling were found to provide enough data to link or discriminate batches of 'ecstasy.

In the future data exchange on 'ecstasy' tablets between European forensic laboratories intelligence must be further developed, if possible with the support from EMCDDA. The

continuous monitoring of the national 'ecstasy' markets and the sharing of data on these tablets will provide a better 'picture' of this dynamic European illicit drug market.

7.3. Seizures of Drugs from EDM Parties and the Malta EDM Party

In Malta the data about illicit substance is dominated by heroin and cannabis and there is no data about 'ecstasy' tablets characterisation. This is because the majority of 'ecstasy' users do not normally come into contact with drug and health agencies and thus there is lack of data on the use of this drug. It is claimed that contrary to heroin, 'ecstasy' tablets are mainly used by young adults as a "club drug" due its popularity and use at parties and clubs [334].

To try and better understand the illicit drug market at EDM parties, especially the 'ecstasy' use, two studies were conducted as described in Chapter 6 of thesis. One of the studies involved the analyses of suspected illicit substances and the characterisation of 'ecstasy' tablets confiscated from eighteen EDM parties in Malta during a six year period (2006 - 2011). The second study, named the "Malta Study", concerned the interviewing of partygoers during an EDM party, in 2010.

The analyses conducted on the seized substances provided information on the drugs used at the EDM parties. This type of drug monitoring, which did not rely on what users thought about their substances, gave an indication of the subtle changes in drug use which was occurring during the period at dance venues in Malta. The most common confiscated drugs from these parties were cannabis, 'ecstasy' tablets and cocaine.

Most of the 'ecstasy' tablets seized at these parties contained MDMA. All the MDMA tablets had a round shape, had a logo and most (78.7%, n = 48 out of 61) were white. The measurable physical features (mass, diameter and thickness) of the MDMA tablets were similar to 'ecstasy' tablets described in Chapter 3 and similar to 'ecstasy' tablets seized in Europe. Thirty of the tablets which were confiscated from the EDM parties had very similar visual and measurable features and dosage to 16 batches (batches 2, 17-19,

21, 24-28, 31, 33, 40, 41, 44 and 45) described in Chapter 3. The rest of the tablets (n = 41) did not match any of the batches. Thus ***hypothesis (5)*** that:

> ***Those 'ecstasy' tablets seized by the police at EDM events in Malta will match batches seized on different occasions in Malta.***

was not verified.

The study on illicit substances confiscated at EDM parties has underlined the importance of on-going monitoring of illicit drug use because of the dynamic nature of this drug market. The changes detected from illicit drug use and drugs habits during monitoring at EDM parties would provide accurate information which could be used by health care professionals to better plan drug prevention intervention. Also, the information gathered about demand side for illicit drugs at EDM parties together with the supply side could provide better data for the market profiling of these drugs.

The results from the "Malta Study", conducted in 2010, indicated no 'ecstasy' use at the EDM event but demonstrated that on the night of the party clubbers were mainly using alcohol, in excessive amounts. The high use of alcohol at this EDM Malta party was found to be similar to alcohol use at similar parties in other countries including Belgium, UK and Australia [218, 242, 329]. From the Malta study it was evident that the three most common illicit drugs 'ever-used' were cannabis, 'ecstasy' and cocaine. From the study of the seizures of illicit tablets from EDM events, it was noted that by 2010 the seizures of 'ecstasy' tablets from parties had decreased. This could have been an indication that either the users of 'ecstasy' were declining at parties, or that the 'ecstasy' use was moving out of parties due to police surveillance, or else new psychoactive substances, like mephedrone, were replacing 'ecstasy' use.

7.4 Future Work

More research work should be carried out on the colour aspect of 'ecstasy' tablets to further confirm that the colour of these tablets could be evaluated by reflectance

spectrometry and to determine the discrimination potential of colour to link or differentiate between batches of tablets. Further work should also be carried out on the stability of organic impurities from MDMA tablets. This would help to determine how MDMA tablets should be stored prior to organic impurity profiling. Another possible future study could focus on the setting up of European computerised database for both physical and chemical characteristics of 'ecstasy' tablets that could be used by European forensic drug laboratories to possibly establish origin of tablets and distribution routes.

Since the number of EDM parties held in Malta has grown and it is accepted that some illicit substance use will continue during these parties, it is contemplated that for the next study the Police Drug Squad section would act as a catchment area to carry out voluntary and confidential interviews with police released partygoers. A pilot study, which was approved by King's College Ethics Committee (PNM/10-11-125, see Appendix 7), to gain information on drug use from low-level offenders has already been conducted. It is hoped that this future study would provide information on changes in patterns of illicit drugs and 'ecstasy' use at EDM parties.

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Appendices

Appendix 1 – Figures of 2-D and 3-D Scatter Plots of Physical Characteristics of 'Ecstasy' Tablets (Chapter 3)

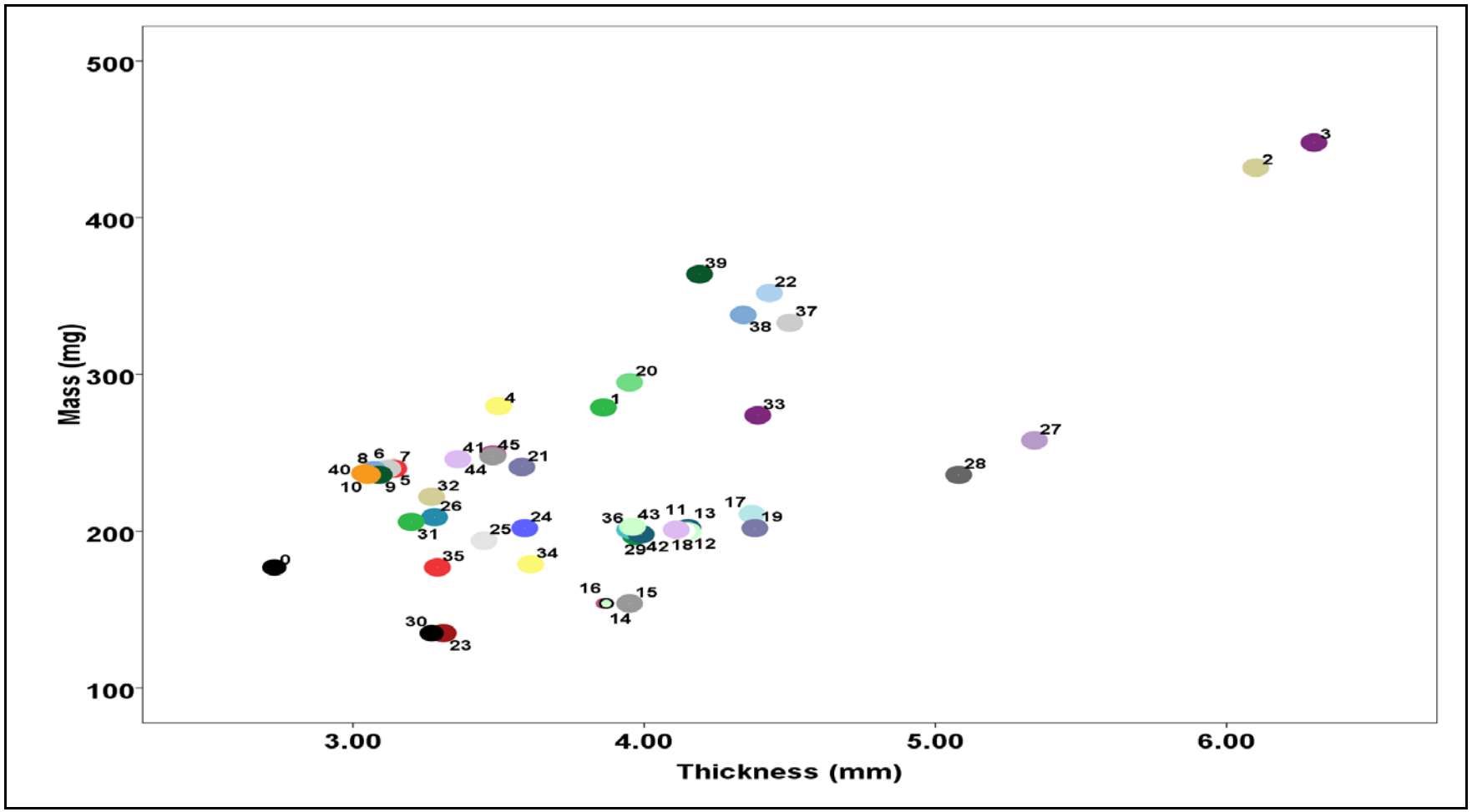

Figure 1.1 2-D scatter plot of the means of mass versus thickness of 45 batches of 'ecstasy' tablets (the numbers near the coloured spots indicate the batch number as in Table 3.2, batch 0 is the control batch, bumetanide).

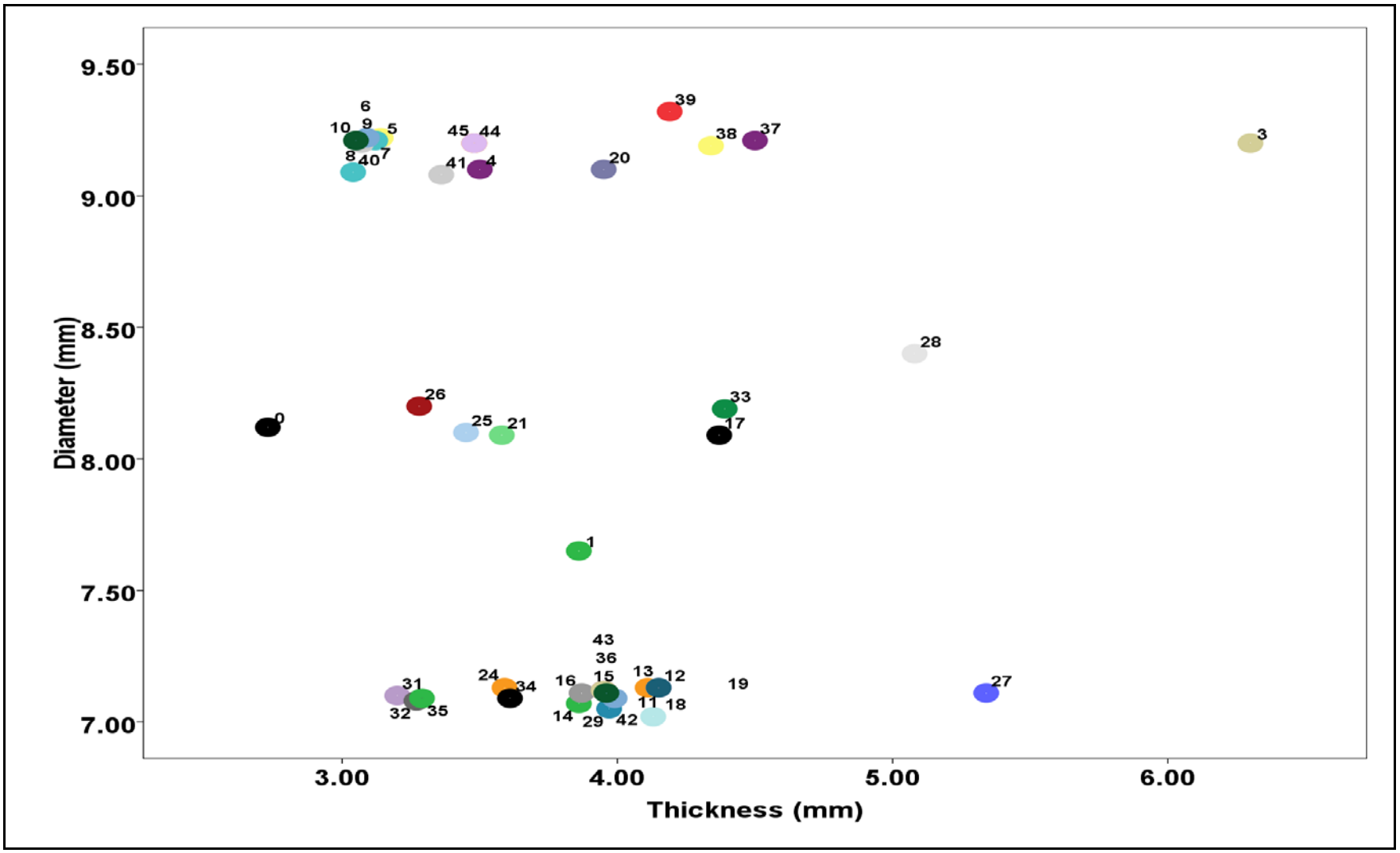

Figure 1.2 2-D scatter plot of the means of diameter versus thickness of 41 batches of round 'ecstasy' tablets (the numbers near the coloured spots indicate the batch number as in Table 3.2, batch 0 is the control batch, bumetanide).

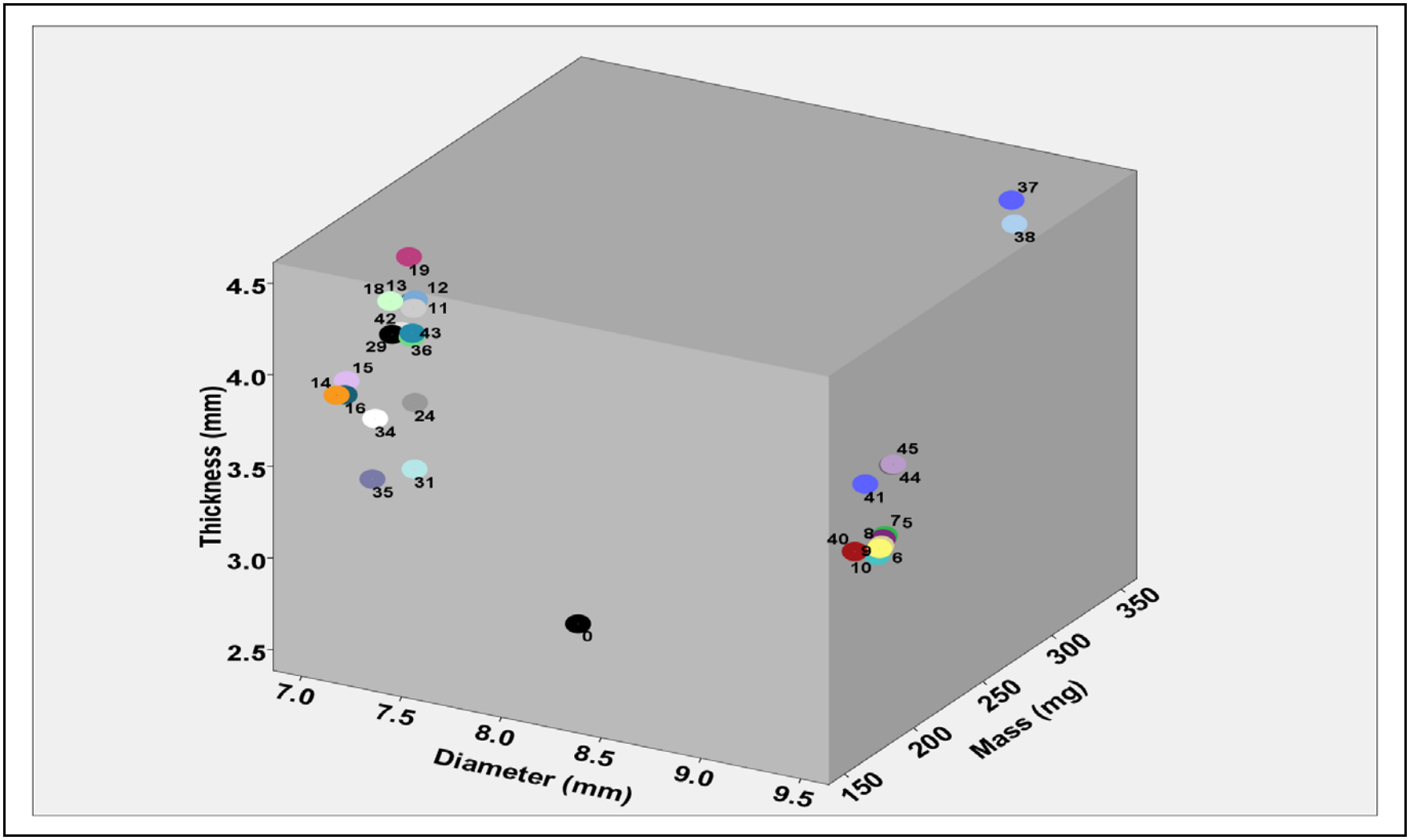

Figure 1.3 3-D scatter plot of the means of mass, diameter and thickness of 28 batches of round 'ecstasy' tablets which appeared touching, overlapping or superimposed in Figure 3.6 (the numbers near the coloured spots indicate the batch number as in Table 3.2, batch 0 is the control batch, bumetanide).

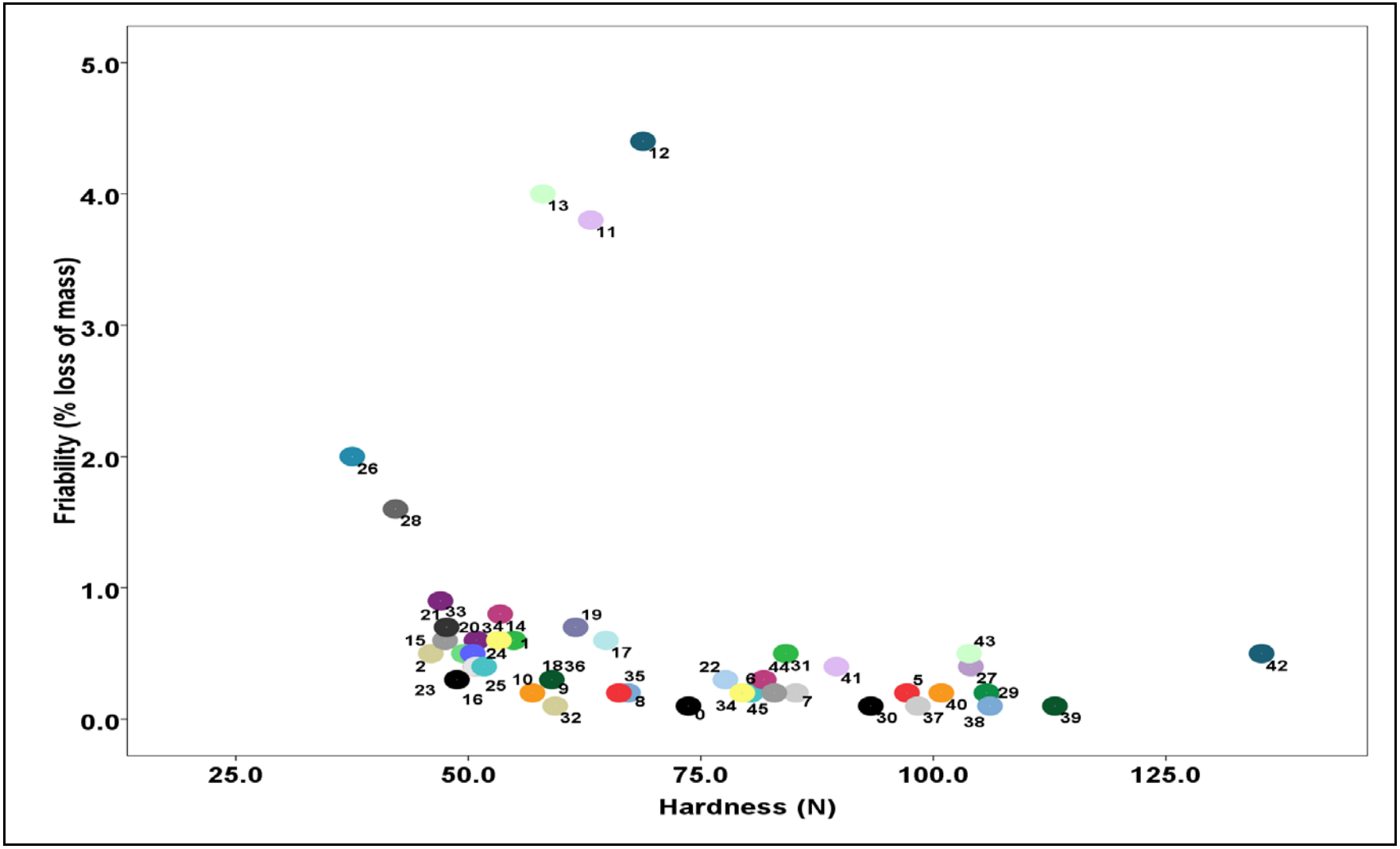

Figure 1.4 2-D scatter plot of the friability versus mean hardness of 45 batches of 'ecstasy' tablets (the numbers near the spots in the scatter plot indicate the batch number as in Table 3.2, batch 0 is the control batch, bumetanide).

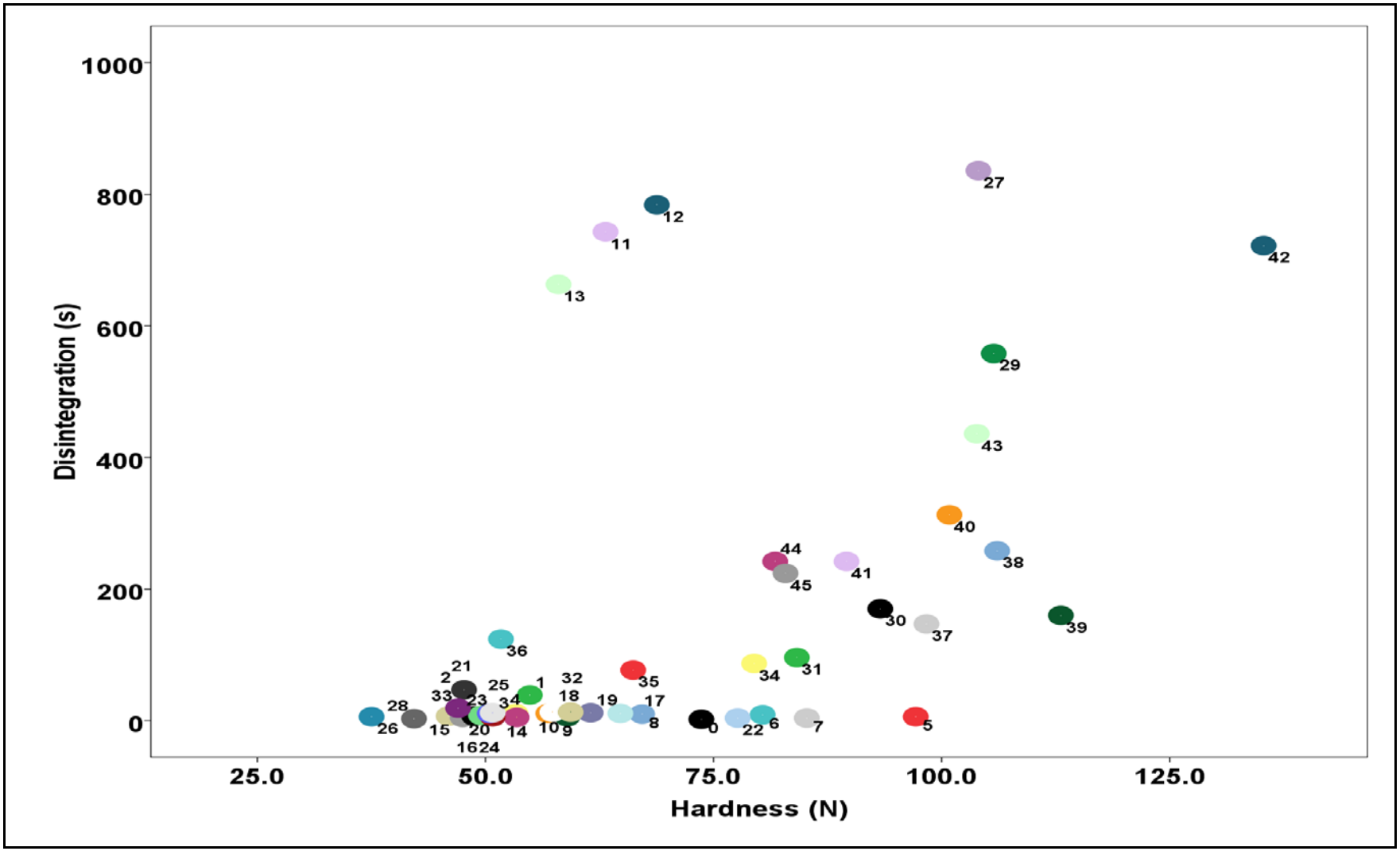

Figure 1.5 2-D scatter plot of the means of disintegration versus hardness of 45 batches of 'ecstasy' tablets (the numbers near the spots in the scatter plot indicate the batch number as in Table 3.2, batch 0 is the control batch, bumetanide).

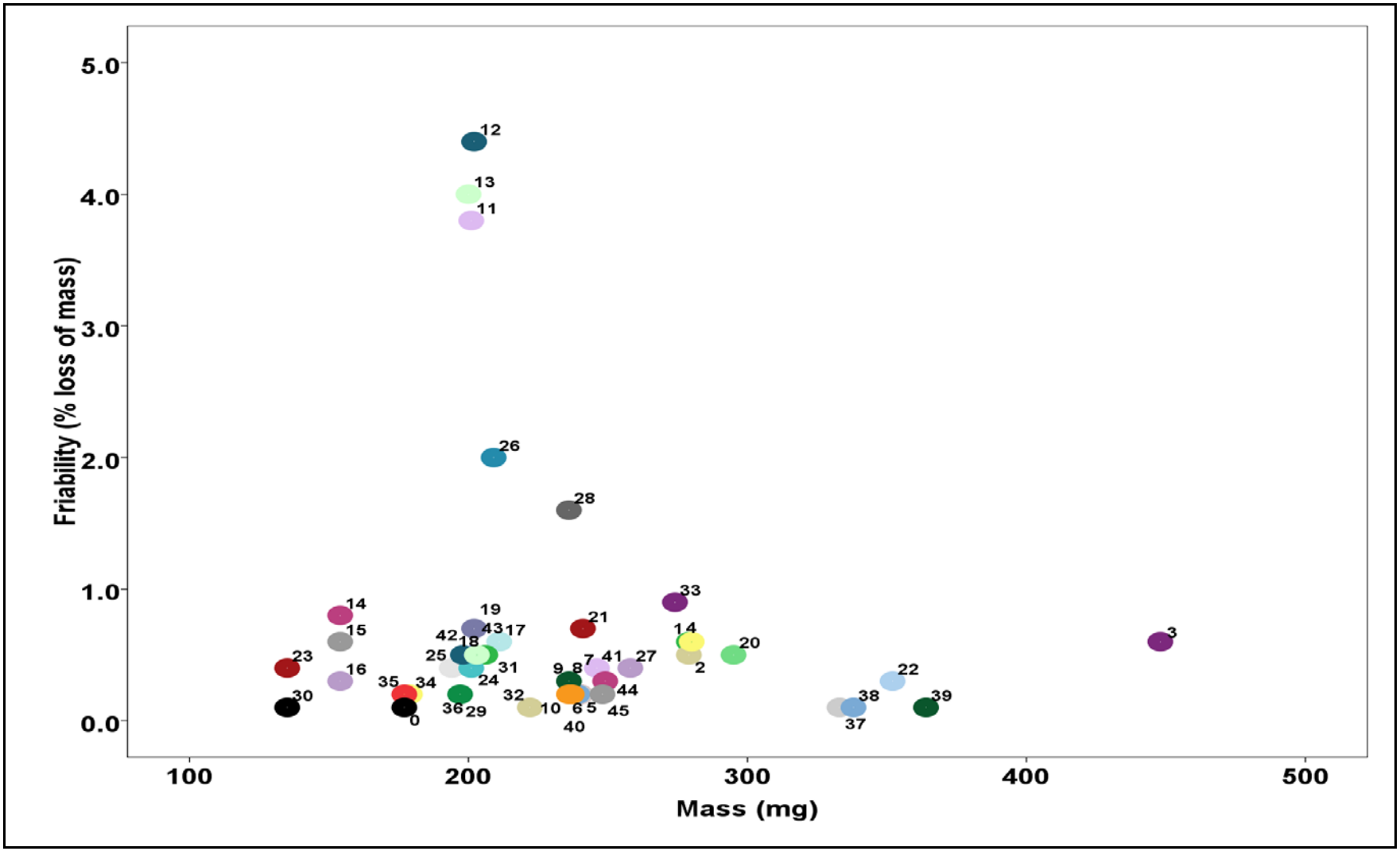

Figure 1.6 2-D scatter plot of the friability versus mean mass of 45 batches of 'ecstasy' tablets (the numbers near the spots in the scatter plot indicate the batch number as in Table 3.2, batch 0 is the control batch, bumetanide).

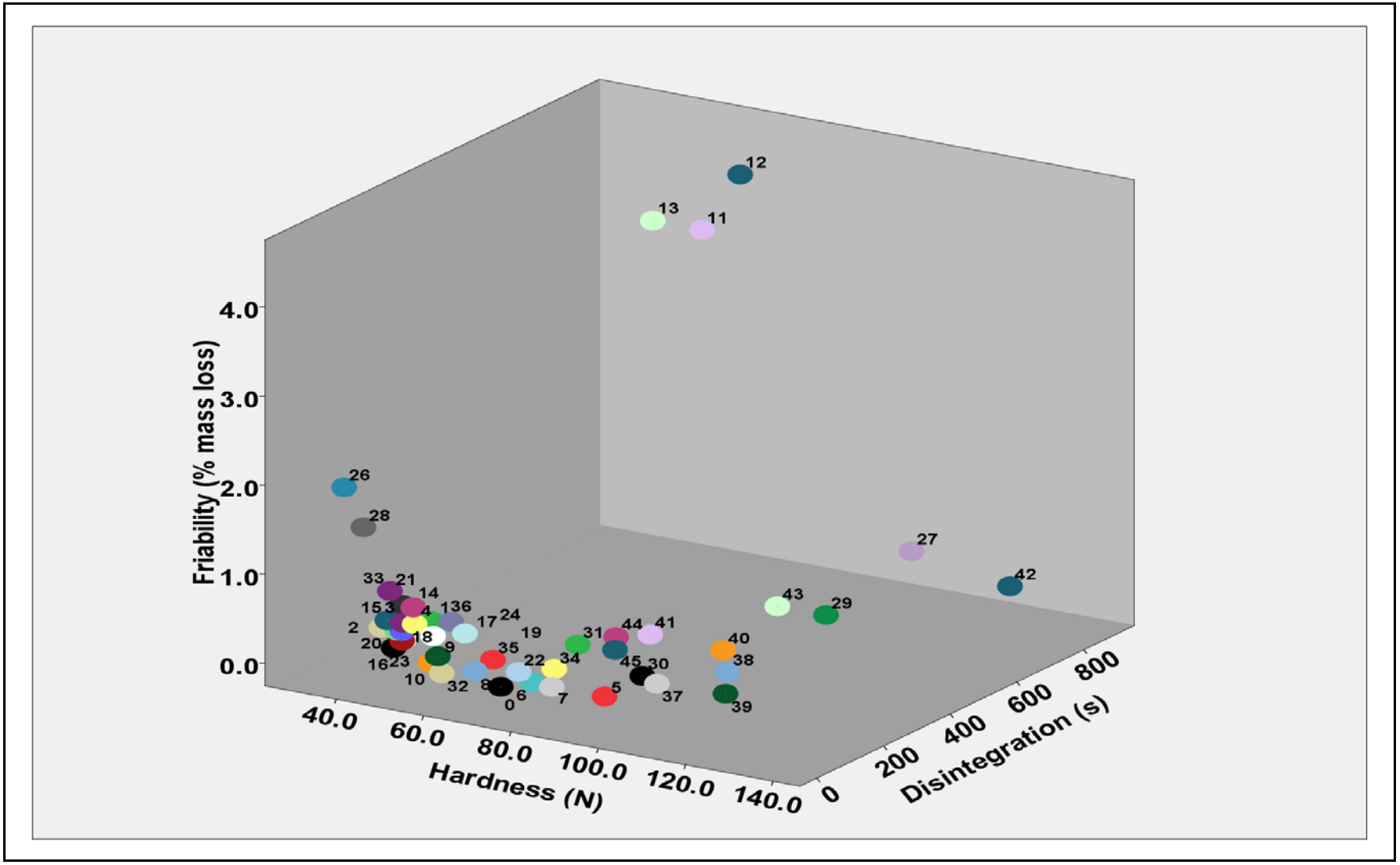

Figure 1.7 3-D scatter plot of the means of disintegration and hardness, plus friability of 45 batches of 'ecstasy' tablets (the numbers near the spots in the scatter plot indicate the batch number as in Table 3.2, batch 0 is the control batch, bumetanide).

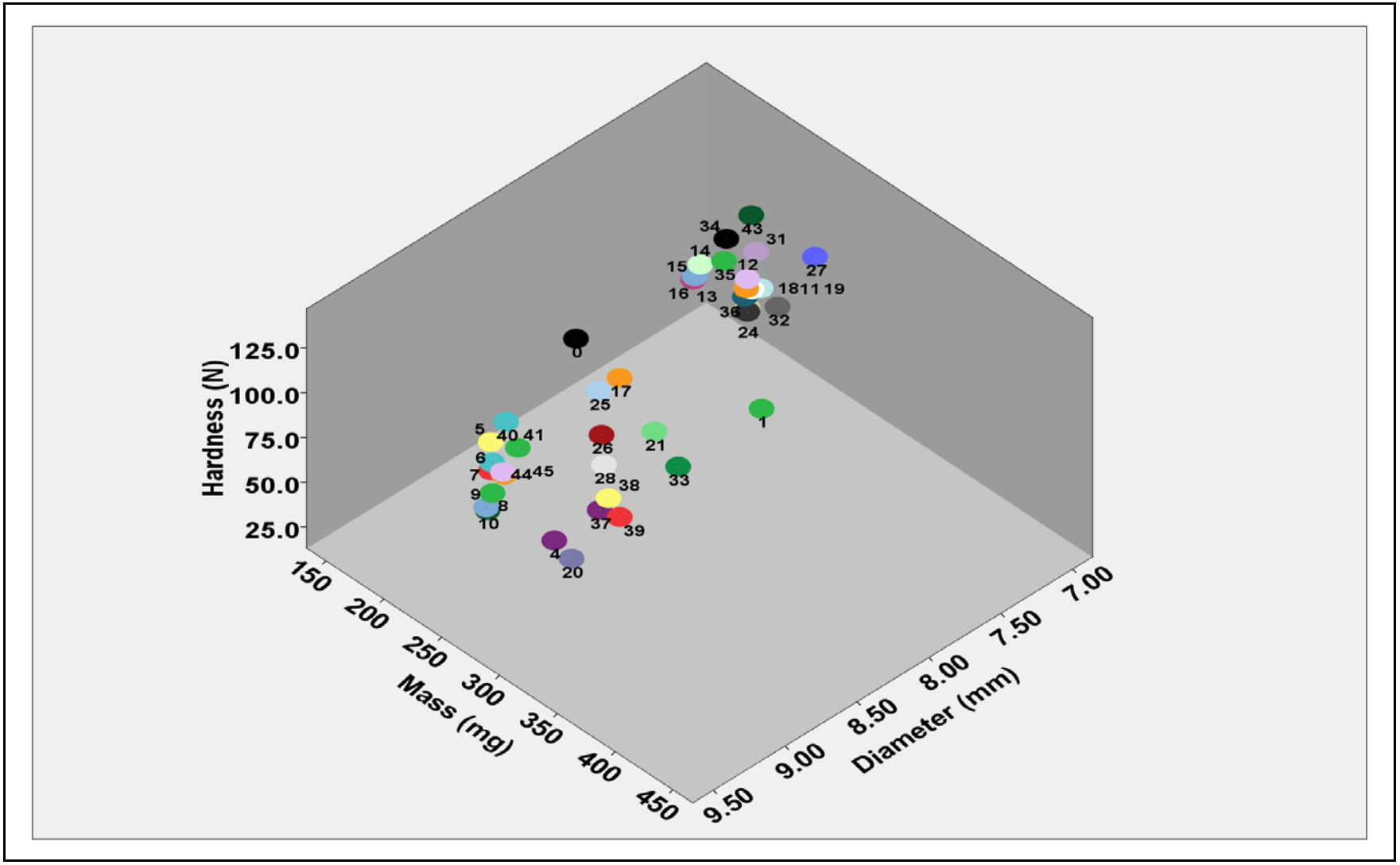

Figure 1.8 3-D scatter plot of the means of diameter, mass and hardness of 41 batches of round 'ecstasy' tablets (the numbers near the spots in the scatter plot indicate the batch number as in Table 3.2, batch 0 is the control batch, bumetanide).

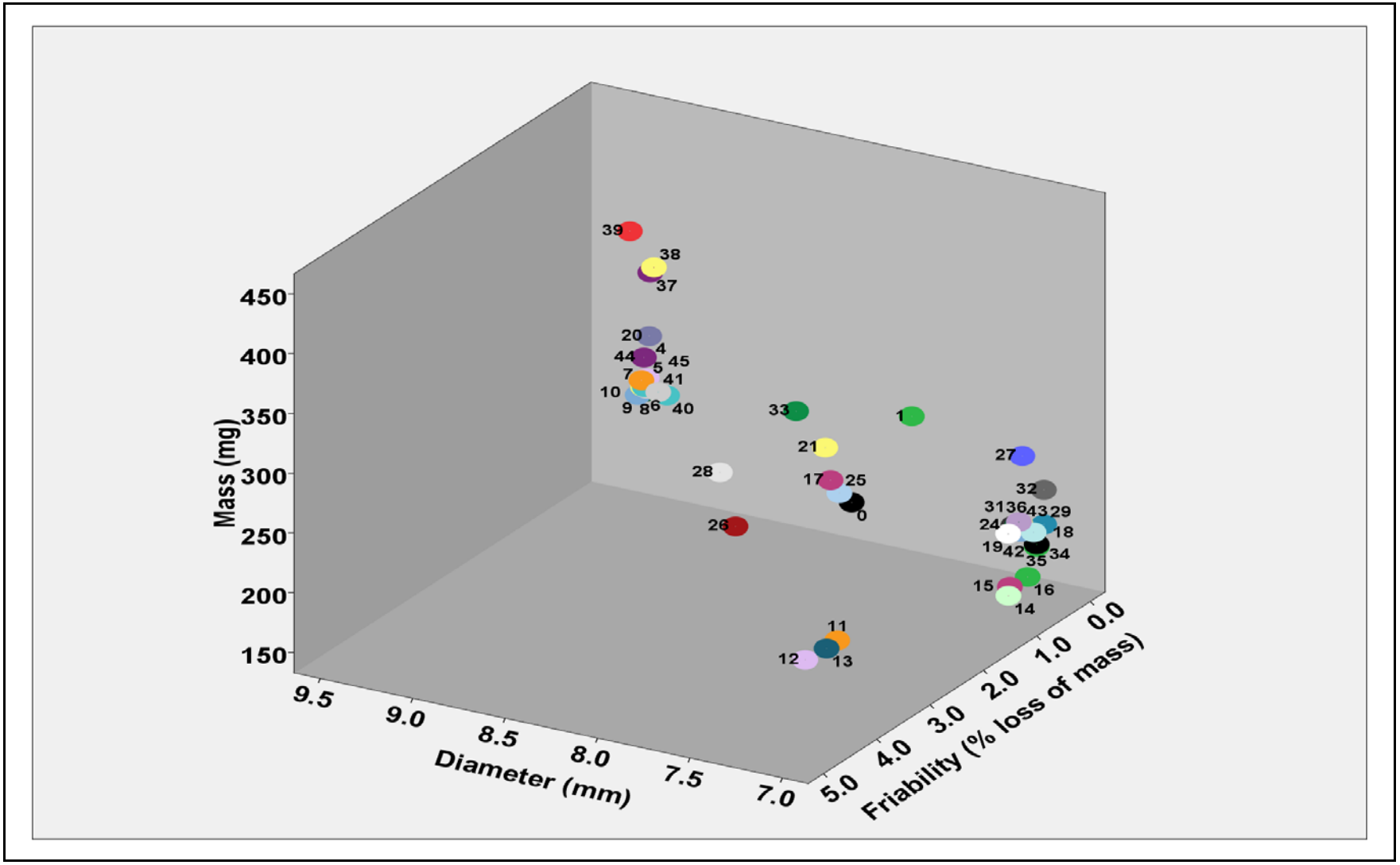

Figure 1.9 3-D scatter plot of the friability and the means of diameter and mass of 41 batches of round 'ecstasy' tablets (the numbers near the spots in the scatter plot indicate the batch number as in Table 3.2, batch 0 is the control batch, bumetanide).

Appendix 2 – Tables of Temperatures and Relative Humidity of Stability Study (Chapter 4)

Table 2.1 Mean mass (mg) of 'ecstasy' tablets when stored at 33 and at 75% RH during the 16 weeks.

RH 33%					
Week	**Temperatures °C**				***p*-value**
	5	**15**	**25**	**35**	
0	270.5 (6.62)	275.1 (7.16)	275.9 (7.02)	277.6 (6.79)	0.141
1	269.6 (6.50)	276.0 (7.45)	273.4 (6.65)	270.3 (6.48)	0.148
2	269.1 (6.52)	277.2 (7.63)	273.2 (6.76)	269.8 (6.32)	**0.045**
3	268.8 (6.58)	277.0 (7.85)	273.1 (6.82)	269.2 (6.55)	**0.041**
4	268.5 (6.52)	276.6 (7.90)	272.5 (6.70)	268.9 (6.44)	**0.044**
5	268.2 (6.76)	276.3 (8.21)	272.1 (6.82)	268.7 (6.48)	0.055
8	268.1 (6.81)	276.3 (8.21)	272.1 (6.82)	268.5 (6.67)	0.051
10	267.7 (6.41)	276.3 (7.96)	271.5 (6.92)	268.6 (6.60)	**0.040**
12	267.6 (6.67)	275.9 (8.05)	271.2 (7.02)	268.2 (6.83)	0.054
14	267.2 (6.73)	275.7 (7.78)	271.0 (6.83)	268.0 (6.91)	**0.045**
16	267.0 (6.93)	275.1 (7.34)	270.9 (6.82)	268.1 (6.52)	0.058
RH 75%					
Week	**Temperatures °C**				***p*-value**
	5	**15**	**25**	**40**	
0	275.2 (6.18)	274.5 (5.68)	273.5 (7.44)	274.0 (5.33)	0.938
1	278.5 (6.50)	278.7 (5.85)	275.1 (7.78)	274.6 (5.56)	0.351
2	277.8 (6.46)	278.4 (5.85)	274.8 (7.64)	273.7 (5.40)	0.298
3	278.1 (6.61)	278.6 (5.85	275.0 (7.66)	274.1 (5.17)	0.316
4	277.6 (6.54)	278.2 (5.98)	274.4 (7.79)	273.3 (5.17)	0.264
5	277.3 (6.57)	277.8 (6.11)	273.9 (8.05)	272.4 (5.06)	0.204
8	277.4 (6.70)	277.9 (6.03)	273.8 (7.91)	271.5 (4.99)	0.106
10	276.7 (6.31)	277.5 (6.02)	273.0 (7.99	271.5 (5.28)	0.134
12	276.5 (6.77)	277.4 (6.04)	272.7 (8.04)	271.3 (4.97)	0.130
14	276.5 (6.06)	276.9 (5.93)	272.4 (8.40)	271.3 (4.97)	0.142
16	276.7 (5.62)	277.0 (6.60)	271.9 (8.60)	271.4 (5.19)	0.122

Key: Tablets from batch 33 stored at different conditions (33% RH and temperatures of 5, 15, 25 and 35°C; 75% RH and temperatures of 5, 15, 25 and 40°C); the numbers in parenthesis are the SDs; the numbers in bold text indicate statistical significance.

Table 2.2 Mean mass (mg) of the total 'ecstasy' tablets (n = 40) when stored at 33 and 75% RH during the 16 weeks.

Week	RH of 33%			RH of 75%			*p*-value
	Mean mass of tablets (mg)	95% Confidence Interval		Mean mass of tablets (mg)	95% confidence Interval		
		Minimum (mg)	Maximum (mg)		Minimum (mg)	Maximum (mg)	
00	274.8 (7.14)	272.5	277.1	274.3 (6.00)	272.4	276.2	0.748
01	272.3 (7.01)	270.1	274.6	276.7 (6.51)	274.6	278.1	**0.005**
02	272.3 (7.32)	270.0	274.7	276.2 (6.46)	274.1	278.2	**0.015**
03	272.0 (7.50)	269.6	274.4	276.5 (6.44)	274.4	278.5	**0.006**
04	271.6 (7.42)	269.3	274.0	275.9 (6.53)	273.8	278.0	**0.008**
05	271.3 (7.57)	268.9	273.7	275.4 (6.69)	273.2	277.5	**0.014**
08	271.3 (7.65)	268.8	273.7	275.2 (6.79)	273.0	277.3	**0.018**
10	271.0 (7.53)	268..6	273.4	274.7 (6.72)	272.5	276.8	**0.025**
12	270.7 (7.64)	268.3	273.2	274.5 (6.80)	272.3	276.7	**0.023**
14	270.5 (7.59)	268.0	272.9	274.3 (6.69)	272.1	276.4	**0.020**
16	270.3 (7.36)	267.9	272.6	274.3 (6.90)	272.0	276.5	**0.015**

Key: Tablets from batch 33 stored at different conditions (33% RH and temperatures of 5, 15, 25 and 35°C; 75% RH and temperatures of 5, 15, 25 and 40°C); the numbers in parenthesis are the SDs; the numbers in bold text indicate statistical significance.

Table 2.3 Mean diameter (mm) of 'ecstasy' tablets when stored at 33 and 75% RH during the 16 weeks.

RH 33%					
Week	**Temperatures °C**				***p*-value**
	5	**15**	**25**	**35**	
0	8.20 (0.02)	8.21 (0.04)	8.20 (0.02)	8.20 (0.02)	0.743
1	8.20 (0.02)	8.21 (0.03)	8.20 (0.02)	8.14 (0.01)	**0.000**
2	8.22 (0.02)	8.22 (0.04)	8.23 (0.01)	8.18 (0.03)	**0.002**
3	8.21 (0.02)	8.21 (0.04)	8.21 (0.03)	8.20 (0.02)	0.718
4	8.22 (0.01)	8.21 (0.03)	8.22 (0.02)	8.20 (0.02)	0.131
5	8.22 (0.02)	8.21 (0.03)	8.21 (0.01)	8.20 (0.02)	0.327
8	8.22 (0.01)	8.22 (0.03)	8.22 (0.02)	8.19 (0.02)	**0.003**
10	8.22 (0.01)	8.22 (0.03)	8.22 (0.02)	8.19 (0.02)	**0.008**
12	8.22 (0.01)	8.22 (0.02)	8.21 (0.02)	8.20 (0.02)	0.071
14	8.22 (0.02)	8.21 (0.02)	8.21 (0.01)	8.20 (0.02)	0.061
16	8.22 (0.02)	8.21 (0.02)	8.21 (0.01)	8.21 (0.03)	0.335
RH 75%					
Week	**Temperatures °C**				***p*-value**
	5	**15**	**25**	**40**	
0	8.19 (0.02)	8.20 (0.03)	8.19 (0.02)	8.20 (0.04)	0.752
1	8.24 (0.02)	8.28 (0.02)	8.27 (0.06)	8.22 (0.02)	**0.001**
2	8.23 (0.01)	8.27 (0.02)	8.24 (0.03)	8.24 (0.02)	**0.005**
3	8.28 (0.03)	8.30 (0.02)	8.25 (0.02)	8.28 (0.03)	**0.007**
4	8.28 (0.03)	8.30 (0.03)	8.25 (0.03)	8.29 (0.03)	**0.003**
5	8.28 (0.02)	8.30 (0.02)	8.24 (0.02)	8.28 (0.02)	**0.000**
8	8.28 (0.03)	8.31 (0.02)	8.27 (0.03)	8.29 (0.02)	**0.007**
10	8.29 (0.02)	8.31 (0.02)	8.25 (0.02)	8.29 (0.04)	**0.000**
12	8.26 (0.02)	8.30 (0.03)	8.25 (0.03)	8.29 (0.03)	**0.000**
14	8.26 (0.02)	8.30 (0.02)	8.25 (0.02)	8.29 (0.04)	**0.000**
16	8.26 (0.01)	8.30 (0.03)	8.24 (0.02)	8.29 (0.04)	**0.000**

Key: Tablets from batch 33 stored at different conditions (33% RH and temperatures of 5, 15, 25 and 35°C; 75% RH and temperatures of 5, 15, 25 and 40°C); the numbers in parenthesis are the SDs; the numbers in bold text indicate statistical significance.

Table 2.4 Mean diameter (mm) of the total 'ecstasy' tablets (n = 40) when stored at 33 and 75% RH during the 16 weeks.

Week	RH of 33%			RH of 75%			*p*-value
	Mean diameter of tablets (mm)	95% Confidence Interval		Mean diameter of tablets (mm)	95% confidence Interval		
		Minimum (mm)	Maximum (mm)		Minimum (mm)	Maximum (mm)	
00	8.20 (0.03)	8.19	8.21	8.19 (0.03)	8.18	8.20	0.175
01	8.19 (0.04	8.18	8.20	8.25 (0.04)	8.24	8.26	**0.000**
02	8.21 (0.03)	8.20	8.22	8.24 (0.02)	8.24	8.25	**0.000**
03	8.21 (0.03)	8.20	8.22	8.28 (0.03)	8.27	8.29	**0.000**
04	8.21 (0.02)	8.20	8.22	8.28 (0.03)	8.27	8.29	**0.000**
05	8.21 (0.02)	8.20	8.22	8.28 (0.03)	8.27	8.29	**0.000**
08	8.21 (0.02)	8.21	8.22	8.29 (0.03)	8.28	8.30	**0.000**
10	8.21 (0.02)	8.21	8.22	8.28 (0.03)	8.27	8.29	**0.000**
12	8.21 (0.02)	8.21	8.22	8.27 (0.03)	8.26	8.29	**0.000**
14	8.21 (0.02)	8.21	8.22	8.27 (0.03)	8.26	8.28	**0.000**
16	8.21 (0.02)	8.21	8.22	8.27 (0.04)	8.26	8.28	**0.000**

Key: Tablets from batch 33 stored at different conditions (33% RH and temperatures of 5, 15, 25 and 35°C; 75% RH and temperatures of 5, 15, 25 and 40°C); the numbers in parenthesis are the SDs; the bold numbers indicate statistical significance.

Table 2.5 Mean thickness (mm) of 'ecstasy' tablets when stored at 33 and 75% RH during the 16 weeks.

RH 33%					
Week	**Temperatures °C**				***p*-value**
	5	**15**	**25**	**35**	
0	4.38 (0.07)	4.39 (0.08)	4.43 (0.08)	4.42 (0.05)	0.477
1	4.36 (0.05)	4.42 (0.08)	4.42 (0.08)	4.40 (0.07)	0.367
2	4.38 (0.04)	4.48 (0.10)	4.41 (0.07)	4.39 (0.07)	**0.033**
3	4.39 (0.05)	4.48 (0.10)	4.42 (0.07)	4.42 (0.07)	0.058
4	4.39 (0.04)	4.48 (0.10)	4.41 (0.06)	4.42 (0.07)	**0.030**
5	4.38 (0.04)	4.47 (0.10)	4.39 (0.07)	4.41 (0.07)	**0.038**
8	4.39 (0.03)	4.48 (0.10)	4.41 (0.05)	4.41 (0.07)	**0.023**
10	4.38 (0.04)	4.48 (0.10)	4.40 (0.05)	4.42 (0.06)	**0.021**
12	4.37 (0.04)	4.47 (0.10)	4.40 (0.06)	4.41 (0.06)	**0.021**
14	4.38 (0.04))	4.47 (0.10)	4.39 (0.06)	4.41 (0.06)	**0.026**
16	4.38 (0.04)	4.46 (0.10)	4.39 (0.06)	4.42 (0.06)	**0.045**
RH 75%					
Week	**Temperatures °C**				***p*-value**
	5	**15**	**25**	**40**	
0	4.37 (0.05)	4.40 (0.09)	4.40 (0.06)	4.38 (0.06)	0.707
1	4.46 (0.08)	4.51 (0.09)	4.46 (0.06)	4.42 (0.06)	0.104
2	4.49 (0.06)	4.54 (0.08)	4.50 (0.05)	4.48 (0.06)	0.118
3	4.48 (0.06)	4.52 (0.09)	4.47 (0.05)	4.48 (0.06)	0.320
4	4.48 (0.07)	4.52 (0.09)	4.47 (0.05)	4.49 (0.06)	0.368
5	4.47 (0.06)	4.51 (0.09)	4.44 (0.04)	4.49 (0.05)	0.165
8	4.48 (0.07)	4.52 (0.09)	4.48 (0.07)	4.51 (0.06)	0.471
10	4.48 (0.07)	4.52 (0.09)	4.48 (0.05)	4.52 (0.06	0.346
12	4.47 (0.07)	4.52 (0.09)	4.48 (0.05)	4.53 (0.06)	0.175
14	4.47 (0.05)	4.52 (0.09)	4.48 (0.05)	4.52 (0.05)	0.239
16	4.48 (0.04)	4.52 (0.09)	4.48 (0.05)	4.52 (0.06)	0.257

Key: Tablets from batch 33 stored at different conditions (33% RH and temperatures of 5, 15, 25 and 35°C; 75% RH and temperatures of 5, 15, 25 and 40°C); the numbers in parenthesis are the SDs; the numbers in bold text indicate statistical significance.

Table 2.6 Mean thickness (mm) of the total 'ecstasy' tablets (n = 40) when stored at 33 and 75% RH during the 16 weeks (Number in bracket is the SD).

Week	RH of 33%			RH of 75%			*p*-value
	Mean thickness of tablets (mm)	95% Confidence Interval		Mean thickness of tablets (mm)	95% Confidence Interval		
		Minimum (mm)	Maximum (mm)		Minimum (mm)	Maximum (mm)	
00	4.41 (0.07)	4.38	4.43	4.39 (0.07)	4.37	4.41	0.267
01	4.40 (0.07)	4.38	4.42	4.46 (0.08)	4.44	4.49	**0.000**
02	4.42 (0.08)	4.39	4.44	4.50 (0.07)	4.48	4.52	**0.000**
03	4.43 (0.08)	4.40	4.45	4.49 (0.07)	4.46	4.51	**0.001**
04	4.42 (0.07)	4.40	4.45	4.49 (0.07)	4.47	4.51	**0.000**
05	4.41 (0.08)	4.39	4.44	4.48 (0.07)	4.46	4.50	**0.000**
08	4.42 (0.07)	4.40	4.44	4.50 (0.07)	4.48	4.52	**0.000**
10	4.42 (0.07)	4.40	4.44	4.50 (0.07)	4.48	4.53	**0.000**
12	4.41 (0.07)	4.39	4.44	4.50 (0.07)	4.47	4.52	**0.000**
14	4.41 (0.07)	4.39	4.43	4.50 (0.07)	4.48	4.52	**0.000**
16	4.41 (0.07)	4.39	4.43	4.50 (0.07)	4.48	4.52	**0.000**

Key: Tablets from batch 33 stored at different conditions (33% RH and temperatures of 5, 15, 25 and 35°C; 75% RH and temperatures of 5, 15, 25 and 40°C); the numbers in parenthesis are the SDs; the numbers in bold text indicate statistical significance.

Table 2.7 Mean volume (mm^3) of 'ecstasy' tablets when stored at 33 and 75% RH during the 16 weeks.

RH 33%					
Week	**Temperatures °C**				***p*-value**
	5	**15**	**25**	**35**	
0	231.5 (4.9)	232.5 (6.2)	233.9 (5.4)	233.3 (3.9)	0.762
1	230.5 (4.1)	234.0 (6.0)	233.2 (5.2)	228.9 (4.2)	0.102
2	232.3 (3.3)	237.6 (7.1)	234.4 (4.7)	230.9 (5.3)	0.123
3	232.6 (3.4)	237.5 (7.4)	234.2 (5.5)	233.4 (4.6)	0.339
4	232.5 (2.7)	237.3 (6.6)	233.6 (4.0)	233.2 (4.5)	0.261
5	232.2 (3.2)	236.8 (7.0)	232.8 (4.1)	232.8 (4.9)	0.367
8	232.6 (2.4)	237.7 (6.6)	234.0 (3.6)	232.5 (4.6)	0.170
10	232.7 (2.8)	237.5 (6.7)	233.4 (3.8)	232.7 (4.4)	0.261
12	232.2 (2.6)	236.9 (6.4)	233.0 (3.9)	233.0 (4.3)	0.248
14	232.3 (2.7)	236.6 (6.2)	232.5 (3.9)	232.7 (4.3)	0.311
16	232.4 (2.9)	236.1 (6.1)	232.1 (3.8)	233.8 (4.8)	0.332
RH 75%					
Week	**Temperatures °C**				***p*-value**
	5	**15**	**25**	**40**	
0	230.4 (3.6)	232.5 (6.6)	231.7 (4.3)	231.1 (5.6)	0.835
1	237.6 (5.3)	242.6 (6.1)	239.8 (6.3)	234.7 (4.5)	**0.024**
2	238.8 (3.9)	243.5 (5.3)	239.5 (4.0)	239.0 (4.5)	0.080
3	241.3 (5.0)	244.5 (6.0)	239.1 (4.2)	240.8 (5.3)	0.148
4	241.0 (5.0)	244.4 (6.1)	238.9 (4.4)	242.0 (4.9)	0.134
5	240.8 (4.6)	243.8 (5.9)	237.0 (3.7)	242.0 (4.2)	**0.019**
8	241.3 (4.7)	245.3 (6.1)	240.9 (5.0)	243.5 (4.2)	0.203
10	241.9 (4.7)	245.0 (5.9)	239.7 (4.3)	244.0 (5.2)	0.106
12	239.4 (4.9)	244.5 (6.2)	239.3 (4.4)	244.2 (5.0)	**0.040**
14	239.6 (3.9)	244.3 (6.2)	239.4 (4.1)	244.0 (5.1)	**0.043**
16	240.0 (2.8)	244.4 (6.4)	239.1 (4.1)	244.2 (5.6)	**0.040**

Key: Tablets from batch 33 stored at different conditions (33% RH and temperatures of 5, 15, 25 and 35°C; 75% RH and temperatures of 5, 15, 25 and 40°C); the numbers in parenthesis are the SDs; the numbers in bold text indicate statistical significance.

Table 2.8 Mean volume (mm^3) of the total 'ecstasy' tablets (n = 40) when stored at 33 and 75% RH during the 16 weeks.

Week	RH of 33%			RH of 75%			*p*-value
	Mean volume of tablets (mm^3)	95% Confidence Interval		Mean volume of tablets (mm^3)	95% Confidence Interval		
		Minimum (mm^3)	Maximum (mm^3)		Minimum (mm^3)	Maximum (mm^3)	
00	232.8 (5.04)	231.2	234.4	231.4 (5.02)	229.8	233.0	0.219
01	231.7 (5.19)	230.0	233.3	238.7 (6.12)	236.7	240.6	**0.000**
02	233.8 (5.66)	232.0	235.6	240.2 (4.72)	238.7	241.7	**0.000**
03	234.4 (5.53)	232.7	236.2	241.4 (5.36)	239.7	243.1	**0.000**
04	234.1 (4.86)	232.6	235.7	241.6 (5.33)	239.9	243.3	**0.000**
05	233.7 (5.17)	232.0	235.3	240.9 (5.13)	239.2	242.5	**0.000**
08	234.2 (4.88)	232.6	235.8	242.7 (5.18)	241.0	244.4	**0.000**
10	234.1 (4.91)	232.5	235.6	242.7 (5.28)	241.0	244.4	**0.000**
12	233.8 (4.73)	232.3	235.3	241.9 (5.56)	240.1	243.6	**0.000**
14	233.5 (4.65)	232.1	235.0	241.8 (5.26)	240.1	243.5	**0.000**
16	233.6 (4.69)	232.1	235.1	241.9 (5.32)	240.2	243.6	**0.000**

Key: Tablets from batch 33 stored at different conditions (33% RH and temperatures of 5, 15, 25 and 35°C; 75% RH and temperatures of 5, 15, 25 and 40°C); the numbers in parenthesis are the SDs; the numbers in bold text indicate statistical significance.

Table 2.9 Hardness (H) of tablets (in Newtons – N) at week 0 and week 16 stored at RHs of 33 and 75% respectively temperatures of 5, 15, 25, 35 and 40°C.

Table Number	Hardness Week 0 (N)	75% RH				33% RH			
		T (^{o}C) – Hardness (N)				T (^{o}C) – Hardness (N)			
		05	15	25	40	05	15	25	35
01	50.49	36.34	34.33	32.59	20 >	52.82	46.12	49.05	49.28
02	48.81	36.16	35.81	33.32	20 >	48.33	47.54	47.63	53.35
03	49.05	38.10	36.46	35.17	20 >	46.92	47.83	56.03	57.47
04	46.55	38.25	35.21	31.57	20 >	43.14	48.89	49.41	51.20
05	47.52	37.68	35.78	32.47	20 >	46.63	49.47	48.32	49.32
06	45.51	35.26	33.92	36.63	20 >	47.12	48.59	48.43	48.47
07	44.76	39.74	36.17	33.13	20 >	45.37	46.35	47.56	45.61
08	45.84	38.45	35.83	32.56	20 >	45.46	43.46	49.26	46.58
09	45.11	38.69	36.67	33.12	20 >	43.11	45.81	48.52	46.11
10	46.35	35.92	34.38	34.83	20 >	46.95	48.53	47.84	48.92
Mean H.	47.00	37.46	35.46	33.54	/	46.59	47.26	49.21	49.63
Min. H.	44.76	35.26	33.92	31.57	/	43.11	43.46	47.56	45.61
Max. H.	50.49	39.74	36.67	36.63	/	52.82	49.47	56.03	57.47

Key: Min. = minimum, Max. = maximum

Table 2.10 Changes in mean mass (mg) of 'ecstasy' tablets from batches 5, 8, 11, after 90 and 180 days when stored at 75% RH and 40°C and tablets from batch 33 after 13 and 16 weeks when stored at 75% RH and at 35 and 40°C.

Tablets	Mean Mass (mg)		Decrease after 90 days / 13 weeks (%)	Mean Mass (mg)	Decrease after 180 days / 16 weeks (%)
	Day 1 / Week 0	Day 90 / Week 13		Day 180 / Week 16	
Batch 5	242 (3.40	241 (3.43)	0.41	239 (3.53)	1.24
Batch 8	239 (4.39)	236 (4.34)	1.26	235 (4.29)	1.67
Batch 11	199 (4.69)	199 (4.63)	0	199 (4.62)	0
Batch 33 (33% RH, 35°C)	278 (2.44)	268 (2.58)	3.60	268 (2.43)	3.60
Batch 33 (75% RH, 40°C)	274 (1.95)	271 (1.86)	1.10	271 (1.92)	1.10
Control	176 (1.71)	176 (1.71)	0	176 (1.71)	0

Key: The numbers in parenthesis are the RSDs

Table 2.11 Changes in mean diameter (mm) of 'ecstasy' tablets from batches 5, 8, 11, after 90 and 180 days when stored at 75% RH and 40°C and tablets from batch 33 after 13 and 16 weeks when stored at 75% RH and at 35 and 40°C.

Tablets	Mean Diameter (mm)		Increase after 90 days / 13 weeks (%)	Mean Diameter (mm)	Increase after 180 days / 16 weeks (%)
	Day 1 / Week 0	Day 90 / Week 13		Day 180 / Week 16	
Batch 5	9.23 (0.43)	9.52 (0.53)	3.14	9.58 (0.52)	3.79
Batch 8	9.21 (0.87)	9.41 (1.06)	2.17	9.49 (0.95)	3.04
Batch 11	7.13 (0.14)	7.19 (0.14)	0.84	7.23 (0.14)	1.40
Batch 33 (33% RH, 35°C)	8.20 (0.24)	8.20 (0.24)	0	8,21 (0.37)	0.12
Batch 33 (75% RH, 40°C)	8.20 (0.49)	8.29 (0.48)	1.10	8.29 (0.48)	1.10
Control	8.16	8.16	0	8.17	0.12

Key: The numbers in parenthesis are the RSDs

Table 2.12 Changes in mean thickness (mm) of 'ecstasy' tablets from batches 5, 8, 11, after 90 and 180 days when stored at 75% RH and 40°C and tablets from batch 33 after 13 and 16 weeks when stored at 75% RH and at 35 and 40°C.

Tablets	Mean Thickness (mm)		Increase after 90 days / 13 weeks (%)	Mean Thickness (mm)	Increase after 180 days / 16 weeks (%)
	Day 1 / Week 0	Day 90 / Week 13		Day 180 / Week 16	
Batch 5	3.14 (4.78)	3.54 (4.24)	12.74	3.68 (3.53)	17.20
Batch 8	3.07 (4.56)	3.27 (3.98)	6.52	3.44 (3.78)	12.05
Batch 11	4.10 (4.15)	4.16 (4.09)	1.46	4.25 (3.77)	3.66
Batch 33 (33% RH, 35°C)	4.42	4.41	0.23 decrease	4.42	0
Batch 33 (75% RH, 40°C)	4.38	4.53	3.43	4.52	3.20
Control	2.77	2.78	0.36	2.78	0.36

Key: The numbers in parenthesis are the RSDs

Table 2.13 Changes in mean volume of 'ecstasy' tablets from batches 5, 8, 11, after 90 and 180 days when stored at 75% RH and 40°C and tablets from batch 33 after 13 and 16 weeks when stored at 75% RH and at 35 and 40°C.

Tablets	Mean Volume (mm^3)		Increase after 90 days / 13 weeks (%)	Mean Volume (mm^3)	Increase after 180 days / 16 weeks (%)
	Day 1 / Week 0	Day 90 / Week 13		Day 180 / Week 16	
Batch 5	210.2 (4.98)	252.0 (4.52)	19.89	264.9 (3.44)	26.12
Batch 8	204.9 (4.92)	227.5 (4.52)	11.03	243.3 (3.80)	18,74
Batch 11	163.8 (4.22)	169.2 (4.11)	3.30	174.5 (3.86)	6,53
Batch 33 (33% RH, 35°C)	233.3 (1.67)	232.9 (1.86)	0.19 decrease	233.8 (2.05)	0.21
Batch 33 (75% RH, 40°C)	231.1 (2.42)	244.1 (2.07)	5.63	244.2 (2.29)	5.67
Control	145.1	145.3	0.14	145.5	0.28

Key: The numbers in parenthesis are the RSDs

Appendix 3 – Sedqa Approval for the Malta Study

sedqa
Aġenzija għall-Ħarsien mill-Abbuż tad-Droga u l-Alkoħol
Agency Against Drug and Alcohol Abuse
Fondazzjoni għal Servizzi ta' Ħarsien Soċjali
Foundation for Social Welfare Services
2-3, Braille Street, Sta Venera HMR 11, Malta

Helpline: 151
Tel: (+356) 2144 1014
Fax: (+356) 2144 1029
www.sedqa.org.mt
sedqa@sedqa.org.mt

Office of the Operations Director

11th August 2010

To Whom It May Concern:

This is to confirm that ***sedqa***, the Maltese national agency again drug and alcohol abuse, has no objection and endorses the research led by Mr. Mario Mifsud in connection with his Ph.D. studies within the Institute of Psychiatry, King's College, London about the consumption of drugs and alcohol by party attendees which is to be carried out in mid-August of 2010.

Jesmond Schembri
Operations Director

Appendix 4 – Consent Form for the Malta Study

Institute of Psychiatry

at the Maudsley

KING'S College LONDON
University of London

CONSENT FORM FOR PARTICIPANTS IN RESEARCH STUDIES

Please complete this form after you have read the Information Sheet and/or listened to an explanation about the research.

Title of Study: Malta's party (rave) scene: a field study on the behaviour of attendees.

King's College Research Ethics Committee Ref:

- Thank you for considering taking part in this research. The person organizing the research must explain the project to you before you agree to take part.
- If you have any questions arising from the Information Sheet or explanation already given to you, please ask the researcher before you decide whether to join in. You will be given a copy of this Consent Form to keep and refer to at any time.
- *I understand that I can decide to withdraw from the study at any time while I am being interviewed.*
- *I consent to the processing of my personal information for the purposes explained to me. I understand that such information will be handled in accordance with the terms of the Data Protection, Chapter 440 of the Laws of Malta.*

Participant's Statement:

I _____

agree that the research project named above has been explained to me to my satisfaction and I agree to take part in the study. I have read both the notes written above and the Information Sheet about the project, and understand what the research study involves.

Signed Date

Investigator's Statement:

I _____

confirm that I have carefully explained the nature, demands and any foreseeable risks (where applicable) of the proposed research to the volunteer.

Signed Date

AUTHORISING SIGNATURES

RESEARCHER

The information supplied above is to the best of my knowledge accurate. I have read the Application Guidelines and clearly understand my obligations and the rights of participants, particularly in so far as to obtaining valid consent. I understand that I must not commence research with human participants until I have received full approval from the ethics committee.

Signature …… Date……..

STUDENT PROJECTS (including PhD) – SUPERVISOR AUTHORISATION

I confirm that I have read this application and will be acting as the student researcher's supervisor for this project. The proposal is viable and the student has appropriate skills to undertake the research. The Information Sheet and recruitment procedures for obtaining informed consent are appropriate and the ethical issues arising from the project have been addressed in the application. I understand that research with human participants must not commence without full approval from the ethics committee.

Name of Supervisor:

Signature …… Date……..

MEDICAL SUPERVISION (if appropriate)

Name of Medical Supervisor:
Medical Supervisor's MDU/MPS (or other insurance provider) number:

……

Signature of Medical Supervisor:

…… Date……..

CONTACT DETAILS Give the details of the individual who should receive all correspondence concerning the application. Correspondence will normally be sent for the attention of the researcher. It is the responsibility of the researcher (and contact if different) to forward all copies of correspondence to the appropriate parties as required. Students should ensure that their supervisor is provided with copies of all correspondence.

Name: Mario Mifsud
Full postal address: 70, Mifriz House, Kittien Street, Zabbar, ZBR 1582.
Telephone number: 21 691917
Email: mifriz@euroweb.net.mt

Appendix 5 – Information Sheet for the Malta Study

Institute of Psychiatry

at the Maudsley

KING'S College LONDON
University of London

INFORMATION SHEET FOR PARTICIPANTS

REC Reference Number: [INSERT ONCE PROVIDED BY REVIEW BODY]

YOU WILL BE GIVEN A COPY OF THIS INFORMATION SHEET

Malta' party (rave) scene: a field study on the behaviour of attendees

We would like to invite you to participate in this postgraduate research project, which is entirely your choice; choosing not to take part will not disadvantage you in any way. Before you decide whether you want to take part, it is important for you to understand why the research is being done and what your participation will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information.

Aims of the study: The aim of the study is to compare the behaviour of attendees at parties (raves) as a function of their choice of substances used in consenting male or female attendees over the age of 18 years. The study will look at information such as age, gender and ethnic group; this is done to understand better who attends raves. It will investigate the party habits of rave attendees. The study will look at participants' choice of access to health care and as well collect information on the consequences of drug use behaviour and on physical and psychological health.

Possible benefits from the study: The potential benefit of this study is that a better understanding will be gained about rave attendees and who goes to these events. Gathering information on drug use behaviour and the effects on general health are very important to be able to create better help for rave attendees. This study will look at what kind of help is sought by raves attendees in case of problems and may help facilitate engagement and access to this population. In general a better understanding of behaviour at rave events will help the development of prevention and harm reduction messages for those who need this information.

If you are happy to participate you will be briefly screened against our exclusion criteria before you enter into the study. We will need to be sure that you are not intoxicated and fully understand the

purpose of the study. The researcher needs to be reassured that you are able answer a number of questions in a competent way. Anyone under the influence of alcohol or drugs and unable to comprehend the information sheet, and, those under the age of 18 years will be excluded.

If you do decide to take part in the study you will be given this information sheet to keep after having completed the interview. It is estimated that completing the interview will take between 10 and 15 minutes.

All subjects who chose to participate in the study will help provide an understanding of the party (raving) environment in Malta by providing crucial data on party (raving) behaviour. On request, all participants can obtain a copy of the completed study for further information.

You may decide to withdraw from the study at any time while you are completing this questionnaire. As there is no identification mark on it, it will not be possible to identify your questionnaire once put with the others. A decision not to take part, will not affect your rights in any way. Anonymity throughout the study is insured. All data will be stored securely and in compliance with the Data Protection Act, Chapter 440 of the Laws of Malta.

You may find that some of the questions are exploring topics or issues that might be sensitive, embarrassing or upsetting, if so do not hesitate to contact us. For any information about drug or alcohol abuse you can Talk to SEDQA on Supportline 179.

Supportline 179

Supportline 179 is a 24 hour free telephone service run by a team of professionally trained volunteers offered by Agenzija Appogg. It provides immediate, confidential support to callers of any age, who require assistance both in day-to-day and crisis situations. It also provides information on social welfare services and referral systems to those callers who require further assistance. Supportline 179 receives calls on situations such as child abuse, domestic violence, drug/alcohol/gambling problems amongst others.

It is up to you to decide whether to take part or not. If this study has harmed you in any way you can contact King's College London using the details below for further advice and information:

Principal investigator: Mario Mifsud

Email: mario.mifsud @kcl.ac.uk

Address: PO Box 048, Addiction Sciences Building, 4 Windsor Walk, Denmark Hill, London SE5 8AF

Appendix 6 – Research Questionnaire for the Malta Study

Institute of Psychiatry

at the Maudsley

KING'S College LONDON
University of London

RESEARCH QUESTIONNAIRE NUMBER: **TIME NOW:**

The researcher confirms that the following observable signs have been considered

Speech: slurring, difficulty-forming words, repetitive, loses train of thought, nonsensical, loud unintelligible? Yes □ No □

Coordination: sways, staggers, stumbles, trips, weaves, walks into objects? Yes □ No □

Appearance: bloodshot-eyes, eyes-glazed, inability to focus, tired, asleep? Yes □ No □

Behaviour: inappropriate actions/ language, rude, aggressive, argumentative? Yes □ No □

Interviewer judged that attendee was able to complete the questionnaire: Y □ N □

I. Demographic information

1. Sex? Male □ Female □

2. Age? _____

3. Ethnic group? White □ Black □ Asian □ Chinese □ mixed □ other_____

4. Nationality? _____

5. Tourist? Yes □ No □

6. English Language School Student? Yes □ No □

7. Current University Student? Yes □ No □

8. Level of Education?

primary level	lower secondary 11+	upper secondary 16+	tertiary level 18+

9. Employment Status?

full-time (≥ 20 hrs/wk)	part-time (< 20hrs/wk)	unemployed

10. Do you live with you parents? Yes □ No □

II. Party Behaviour

1. How often do you party? **every week** **2 to 3 times/month** **once monthly or less**

2. With whom do you usually go to a party?

alone **with partner** **with friend** **group of friends n=_____**

3. Typically <u>before</u> a party do you do any of the following? Take the day off work □

Not drink any alcohol □ Have a good night sleep □ Not use any drugs □

Have a good meal □ Let friends know you're going out □ Take vitamins or minerals □

4. Typically how long do you party for in one go? _____hours

5. At a party how much of the time is spent dancing?

almost none **20%** **40%** **60%** **80%** **most of the time**

6. Do you go to after parties?

never **seldom** **sometimes** **often** **always**

7. How much money do you spend on a typical night out? _____EUR

8. Do you drink any <u>non alcoholic</u> drinks at a typical party?

Water □ Soda □ Fruit juice □ Sport drink □ Energy drink □

Other?_____

How much?_____

What?_____

9. How many alcoholic drinks do you have at a typical party?

How much?_____What?_____

10. AUDIT-C	**Scoring system**					**Your score**
	0	**1**	**2**	**3**	**4**	
How often do you have a drink containing alcohol?	Never	Monthly or less	2 - 4 times per month	2 - 3 times per week	4+ times per week	
How many units of alcohol do you drink on a typical day when you are drinking?	1 -2	3 - 4	5 - 6	7 --9	10+	
How often have you had 6 or more units if female, or 8 or more if male, on a single occasion in the last year?	Never	Less than monthly	Monthly	Weekly	Daily or almost daily	

III. Drug Consumption

Substances	ever used	age first used	used this year	days used last month	used today
Alcohol					
Tobacco					
Cannabis resin (hasish)					
Cannabis – oil					
Cannabis – grass					
Cannabis – skunk					
LSD/acid					
Magic mushrooms					
Cocaine (powder)					
Crack cocaine					
Amphetamine Powder (speed)					
Methamphetamine (crystal meth)					
Ritalin					
Opium					
Heroin					
Methadone					
Benzodiazepines (eg. Vlalium/diazepam, Librium)					
Ketamine					
alkyl nitrate (poppers)					
GHB (*Gamma*-Hydroxybutyric acid)					
GBL (*gamma*-Butyrolactone)					
Anabolic steroids					

Substances	**ever used**	**used this year**	**age first used**	**days used last month**	**Used today**
Nitrous Oxide (laughing gas)					
spice / magic / warrior					
BZP (Benzylpiperazine - Party Pills)					
Mephedrone (meow)					
MDAI (woof woof)					
Methylone					
Salvia Divinorum					
Viagra (Cialis)					
herbal ecstasy / ephedra					
Ecstasy tablets					
MDMA (ecstasy powder)					
Other..					

1. Do you take any prescribed medication? What?

_____

2. Do you always use drugs at a party?	**never**	**seldom**	**sometimes**	**often**	**always**

3. What drugs do you most commonly use together during a party (incl route)? a. ***alcohol*** b. ***cannabis*** c. ***nicotine***

d._____e._____f._____g__.__

4. PILLS: Please describe what you have taken today: Name?_____How many? _____Shape?_____

Colour?_____ Logo_____How much did it cost?_____

5. POWDER: Please describe what you have taken today: Name?_____How much?_____

Texture?_____Colour?_____How much did it cost? _____Route?_____

IV. Use of Ecstasy

IF ECSTASY AND/OR MDMA HAS BEEN USED IN THE LAST MONTH PLEASE ANSWER THE FOLLOWING

1. **What is the average number of tablets you take during a party?**_____

2. **What is the greatest number of tablets you have ever taken during a party?** _____

3. **What do you think about the quality of ecstasy tablets during the last 6 months?**

gone up **gone down** **stayed the same** **don't know**

4. **What is the most number of consecutive days you have used ecstasy?** _____

5. **Does it matter to you whether or not ecstasy tablets contain MDMA?** Yes □ No □

5a. Why?_____

6. **Do you ever 'test' your ecstasy tablets?**

never **seldom** **sometimes** **often** **always**

6a. How?_____

7. **If you could, would you like to test your 'ecstasy' tablets?** Yes □ No □

7a. Why? Health concern □ Curiosity □ Check dealer □ Other?_____

8. **How many tablets do you usually buy and how much do they cost?**

_____

9. **Do you ever take MDMA (powder)?** Yes □ No □

10. **Do you prefer tablets, or MDMA (powder)?** tablets □ powder □ no preference □

10a. Why? easier to dose □ better quality □ easier to get hold of □ other_____

11. **How much MDMA do you buy and how much does it cost?**_____g_____EUR

12. Typically do you take any of the following drugs with Ecstasy?

12a. Antidepressants? SSRIs eg Prozac, Seroxat, Sertraline, Lustral, Sertralin, Sertral

Yes □ No □ What?_____

Is this prescribed?	Yes □	No □	**before**	**after**	**at the same time**
12b. Vicks inhalants	Yes □	No □	**before**	**after**	**at the same time**
12c. Viagra (sextasy)	Yes □	No □	**before**	**after**	**at the same time**
			before	**after**	**at the same time**
12d. LSD (candy-flipping)		Yes □ No □			
12e. Ketamine (kitty-flipping)		Yes □ No □	**before**	**after**	**at the same time**
12d. Mushrooms (hippy-flipping)		Yes □ No □	**before**	**after**	**at the same time**

12. How often do you take the following substances when you use Ecstasy?

13a. Alcohol	**never**	**seldom**	**sometimes**	**often**	**always**
13b. Cannabis	**never**	**seldom**	**sometimes**	**often**	**always**
13c. Cocaine	**never**	**seldom**	**sometimes**	**often**	**always**
13c. Mephedrone	**never**	**seldom**	**sometimes**	**often**	**always**

14. After taking ecstasy or MDMA do you typically experience any of the following?

excessive sweating □ thirst/dehydration □ numbness/ tingling □ vomiting or nausea □

feeling dizzy □ sexual problems □ unable to urinate □

anything we have missed?

V. Post Party Behaviour

1. At a typical party do you become unwell?

never	seldom	sometimes	often	always

1a. Why might this be?_____

_____

2. How long do you usually sleep after a party?

less than 6 hours	6 to 8 hours	8 to 12 hours	more than 12 hours

3. Do you take extra fruit or vitamins the day after a party?

never	seldom	sometimes	often	always

3a. What?_____ _____

4. Do you take anything to help you sleep after a party?

never	seldom	sometimes	often	always

4a. What? _____

Thanks for your collaboration

PLEASE GIVE CARD TO THOSE WHO WANT FURTHER INFORMATION OR WOULD LIKE TO PARTICIPATE IN FURTHER RESEARCH

Appendix 7 – King's College Ethics Committee Approval for Pilot Study for Police Released Partygoers

Mario Mifsud
Institute of Psychiatry
Box PO 48
Addiction Sciences Building
De Crespigny Park
SE5 8AF

12 September 2011

Dear Mario

PNM/10/11-125 Behaviour of Police Released Detainees found in Possession of Ecstasy and Other Clubbing Drugs (ECOD)

Thank you for sending in the amendments requested to the above project. I am pleased to inform you that these meet the requirements of the PNM RESC and therefore that full approval is now granted.

Please ensure that you follow all relevant guidance as laid out in the King's College London Guidelines on Good Practice in Academic Research (http://www.kcl.ac.uk/college/policyzone/index.php?id=247).

For your information ethical approval is granted until **12 September 2013**. If you need approval beyond this point you will need to apply for an extension to approval at least two weeks prior to this explaining why the extension is needed, (please note however that a full re-application will not be necessary unless the protocol has changed). You should also note that if your approval is for one year, you will not be sent a reminder when it is due to lapse.

If you do not start the project within three months of this letter please contact the Research Ethics Office. Should you need to modify the project or request an extension to approval you will need approval for this and should follow the guidance relating to modifying approved applications: http://www.kcl.ac.uk/research/ethics/applicants/modifications.html

Any unforeseen ethical problems arising during the course of the project should be reported to the approving committee/panel. In the event of an untoward event or an adverse reaction a full report must be made to the Chairman of the approving committee/review panel within one week of the incident.

Please would you also note that we may, for the purposes of audit, contact you from time to time to ascertain the status of your research.

If you have any query about any aspect of this ethical approval, please contact your panel/committee administrator in the first instance (http://www.kcl.ac.uk/research/ethics/contacts.html). We wish you every success with this work.

With best wishes

Yours sincerely

Jim Summers
Research Ethics Team Leader

c.c. Dr Kim Wolff